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HEREDITY VOLUME 8 PART 2 AUGUST 1954

THE LACK OF PROPORTIONALITY BETWEEN MUTATION RATE AND ULTRAVIOLET DOSE IN *DROSOPHILA* *

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MARGARET EDMONDSON,‡ and EDGAR ALTENBURG †

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1. INTRODUCTION

ALTHOUGH mutagenesis by X and gamma radiation is effected by individual "hits", these hits may comprise clusters of ionisations, and each ionisation may itself undergo degradation to clusters of excitations and of other effects involving lesser energy, and of varied kinds. As ultraviolet light, on the contrary, usually results in individual excitations, independently produced, but largely concentrated within given types of materials, it would seem in some respects better suited for the analysis of mutagenic phenomena. Any studies utilising it for this purpose should, however, proceed on the basis of information gathered in preliminary investigations, in which data have been obtained regarding the manner in which the production of mutations, and the recovery by the investigator of the mutations produced, is influenced by change in dose, and by change in the conditions of applying the dose. In the series of studies here to be reported some of these questions have been investigated in our material.

2. MATERIALS AND METHODS OF TREATMENT

Although much work has been reported on the relation between the mutation rate and dosage of ultraviolet, most of this has been done on microorganisms, and it was therefore thought desirable to investigate the problem further with *Drosophila* because of the unusual suitability of this material for the genetic analysis of the results.

However, the adult *Drosophila* is not very transparent to ultraviolet light. Only a very small fraction of ultraviolet that strikes the surface of an adult male can penetrate as deeply as the gonad. In order for appreciable amounts of ultraviolet to reach the germ cells of the adult fly, it is therefore necessary to use excessive doses, and these are highly damaging to the individual. Moreover, when the adult fly is treated, it is advisable to compress the abdomen between two plates, both to hold it in place during treatment and also to bring the gonads (testes, since oogonial and oocyte nuclei are still harder to reach) closer to the surface. As the degree and type of compression unavoidably vary somewhat from fly to fly, as well as the transparency of its surface layers to the ultraviolet, there is considerable uncontrollable variation in the dose that gets to the germ cells. Hence it is impracticable, for quantitative experiments on ultraviolet dosage, to treat the adult males, as is customary with X-rays. Recourse was therefore had to ultraviolet treatment of embryos, according to the technique first applied by Geigy (1931) and developed

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further, with special reference to mutation frequency studies, by E. Altenburg (1933-36).

At a certain stage in the development of the fertilised egg—the " polar cap " stage—the cells of the early germ track of *Drosophila* are located just below the vitelline membrane, at the amicropolar end of the egg, where they form a cap of cells (the polar cap, fig. 1). When the chorion (or shell) of the egg is removed, these polar cap cells are separated from the outside surface by only the thin, transparent vitelline membrane and thus are almost directly exposed to any ultraviolet light that strikes the overlying surface. Moreover, the polar cap cells are now readily visible under the microscope, so that it is possible to examine the eggs just before or after treatment and see whether or not they are in the polar cap stage. In the present experiments, only eggs from which the shells were removed (dechorionated eggs) were treated. Moreover, the eggs were examined under the microscope just before (Rice group) or just after (Indiana group) treatment, and any not in the polar cap stage were rejected. In addition, in the post-treatment examinations, those eggs, often constituting a majority of the total, were rejected which already showed visible signs of injury, such as vacuolisation and interruption of embryonic development, since these were found by experience to be in the great majority of cases moribund.

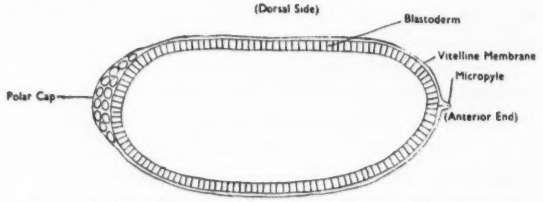

FIG. 1. A *Drosophila* egg at the polar cap stage of development, with chorion removed.

The polar cap stage sets in about two hours and fifteen minutes after fertilisation, at 25° C., and it lasts for about seventy minutes (see, for instance, the excellent summary by Sonnenblick, 1950). Most of the polar cap cells then migrate into a pocket, the proctodeal invagination, located under the dorsal surface of the egg just a little distance in front of their original position. Once in the pocket they are well below the surface of the egg and no longer directly accessible to ultraviolet light. Treatments must therefore be completed before the polar cap stage ends.

At 2½ to 3 hours after the beginning of development, the budding of cells into the polar cap from the underlying syncytium has ceased ; the blastoderm has been formed and its nuclei have taken on a characteristic elongated shape, and the polar cap cells are no longer found in division. There are then some 55 (37 to 73, according to Rabinowitz, 1941) pole cells present. Rabinowitz states that some of these migrate into the yolk, during the polar cap stage, and there disintegrate. When the pocket is formed, only some 30 or 40 pass into it, and finally only 10 to 26 of these, about half as many in the male (10 to 14) as in the female (18 to 26), become incorporated (without any intervening mitosis) in the gonads (Sonnenblick, 1941). As Poulson (1947) has shown, some of the pole cells which enter the pocket but do not get into the gonads take part in the formation of the midgut. At 16 hours after fertilisation, some 6 hours after the establishment of the gonads, cell division of the germ cells is resumed (Sonnenblick, 1941).

Any mutant gene originating in a chromosome of a polar cap cell is multiplied, in the course of mitotic cell division, to the same extent that the cell multiplies. Such mutants therefore are often found in " clusters ". Thus, for example, if the sperm cells of the mature male were derived from five polar cap cells, in one of which a lethal was induced, and if all these polar cap cells had multiplied equally,

then about $\frac{1}{8}$ of the sperm cells with the kind of chromosome in question would contain the lethal. Thus if 10 such chromosomes had been tested for lethals, $\frac{1}{8}$ of these 10, on the average, or a cluster of 2, would contain the lethal. These two lethals would be at the same locus, and a test for their allelism would give positive results. By contrast, two lethals of independent origin, derived from two different cells of a polar cap, would most likely be at different loci, and hence an allelism test would prove negative.

Recent results on mutant cluster size obtained at Indiana University, in tests of some 37 second chromosomes of a given genome per polar cap, after an ultraviolet dose low enough to give only about 5 per cent. of second chromosome lethals, have indicated that about 13 pole cells proliferate to form spermatozoa, since on the average a given lethal was found in $\frac{1}{13}$ (7·6 per cent.) of all the tested chromosomes derived from the same genome of the same treated polar cap. This figure agrees well with the previously mentioned one of 10 to 14 based on cell counts of the primordial testis. It can be shown mathematically that even when the different cells of each polar cap proliferate to different extents, so as to give rise to clusters of different sizes, the average cluster size, obtained by dividing the number of lethals found by the number of clusters they were in and then dividing this quotient into the average number of chromosomes tested per genome of a polar cap, is still the reciprocal of the total number of germinally functioning cells per polar cap.

TABLE 1

Increase in Size of "Runs" with Age of Father

Treatment			Imaginal age of P_1 ♂ in weeks	No. of second chrom. tested	No. of lethal second chrom.	Per cent. of lethal chrom.	Average size of runs (in per cent.)
Time (min.)	Distance	Relative dose					
3	50 cm.	14	0-1	180	35	19·5	29·3
			1-3	213	27	12·7	41·0
6	150 cm.	3·6	0-1	173	35	20·2	23·5
			1-3	184	28	15·2	46·9

This only holds, however, in situations in which it can be assumed that the pole cells containing the lethals found have not had their multiplication materially depressed below that of those cells from which no lethals were obtained, for if they had been more retarded the relative size of the clusters derived from them would be reduced and the calculated number of pole cells would thereby be rendered larger than the actual number. A test of whether this was the case in our experiments which indicated some 13 pole cells would be obtained by determining whether or not, with still further decrease of the dose, the cluster size became larger again, as it would if a part of the small size had been caused by selective physiological damage.

Proceeding in the other direction, that of increase of dose, our experiments show that at a dose high enough to produce as many as 10-20 per cent. of second chromosome lethals in the sperm of the first week after hatching, the clusters are considerably larger, forming one-fifth to one-fourth of the total, as would be the case if only 4-5 or fewer pole cells had proliferated to give rise to gametes. In general, our results on the size of clusters show that the higher the dose, the more primary germ cells have been inactivated, as might be expected from the killing action of the ultraviolet.

The number of pole cells which act as progenitors to sperm released after the first week becomes more limited as time passes. This was shown by the results of Harris (1929), working under Muller's direction, on the large clusters of lethals (averaging about 25 per cent. of the total) in sperm released some three weeks after X-raying of the adults, and by the similar results of Friesen (1936) on the clusters of X-ray induced crossovers in such sperm. These results lead to the conclusion that there are usually only about two cells per testis (four per individual) which, like apical cells, continue indefinitely to give rise, by their proliferation, to the end stages, the spermatozoa. When, however, large enough doses of ultraviolet have been given to reduce to four or five the number of pole cells which give rise to the spermatozoa of the first week, a still smaller number of these pole cells, namely only 2 to 3 per individual, is represented among the spermatozoa of the second and third weeks. Table 1 gives results obtained at Indiana University, which illustrate this relation. The word " run " is here used in place of " cluster ".

The same males were used as fathers in weeks 1 to 3 as in week 0 to 1, and the lethals obtained from the later sperm proved, as expected, to be of identical origin (allelic to) those from the earlier sperm (except in the case of a single later lethal). However, not all the lethals of the earlier sperm reappeared in the later sperm although those that did were present in larger " runs ". Approximately 10 second chromosomes were tested per genome (20 per male) in the first week, and somewhat more in the combined second and third weeks. The first week's offspring here reported upon also form a part of the material given in table 4. Those males were purposely chosen for the tests of the later weeks which, according to preliminary fertility indications, would give a higher than average per cent. of lethals in the first week. Thus there was in the later weeks some regression of the rate, caused by a portion of the apparently greater early effects having arisen from the selection of those random fluctuations in the time distribution of the effects which happened to be in the direction of a higher early rate. The observed drop in rate with age must therefore be, in part at least, spurious. The fact that selection was used also makes the material unreliable for a comparison between mutation rates produced by different doses. However, this influence does not explain the increase in the size of runs with age since it would work in the opposite direction, inasmuch as selection in the first week for higher rate would include selection for larger runs. This makes the observed increase in size of runs with age of father the more significant, and shows that the later sperm are on the average derived from fewer pole cells than are the earlier sperm.

It follows from the increase in cluster size with age that when sperm of the first week are used a larger proportion of treated pole cells is sampled than when late sperm are used. Since larger samples give rise to smaller statistical errors, we therefore made it a practice to use sperm of the first week so far as practicable, although when a young male did not yield enough offspring these were sometimes supplemented by those derived from the same male in its second week.

Ultraviolet light is particularly damaging to a *Drosophila* egg (embryo) at the polar cap stage of development. If the entire egg is treated, the dose of ultraviolet that can be tolerated is not sufficient to induce a mutation rate much above the spontaneous rate. In the present experiments, therefore, the egg as a whole was shielded from the ultraviolet and only the polar cap exposed. In the earlier experiments this was done by placing the eggs along the sharply cut edge of a moist blotter or along the edge of a piece of tinfoil, with little but the polar caps projecting beyond the edge, into the region illuminated by the ultraviolet (fig. 2*a*).

However, by this method, as at first used, the rays of the ultraviolet are at right angles (90°) to the long axis of the egg, striking the polar cap from one side (at about 15° to its own plane) so that the polar cap cells on the further side are very much in the shadow of those first struck. This uneven treatment must cause the mutation rate at the higher doses to appear lower than it actually is, since the ultraviolet must selectively kill the cells which receive the highest treatment and

have the highest rate. A second method for shielding the eggs was therefore used in some of the later experiments at Rice Institute. This consisted in putting the polar cap ends of the eggs into small holes in a piece of aluminium foil, so that only the polar cap projected through the hole and was exposed to the ultraviolet light (fig. 2*b*). By this method the ultraviolet is parallel to the long axis of the egg and strikes the plane of the polar cap, which is slightly tilted, almost from above, *i.e.* at about 75°. It therefore strikes the cells much more uniformly than it does in the case of the first method, by which the polar cap is treated from the side. In the experiments here reported which were carried out at Indiana University, although the first method of shielding was used, the light was caused to fall at an angle of 45° to the egg axis, or at about 60° to the plane of the polar cap (fig. 2*c*).

The source of the ultraviolet light in the present experiments was a 15-watt G.E. germicidal lamp, most (over 90 per cent.) of the ultraviolet radiation of which is of 2537 Å wave length. This is close to the region of the ultraviolet spectrum which is most highly absorbed by nucleic acid and which is most efficient in the production of mutations. However, the lamp itself is far from being a point source of radiation, since the luminous portion of the 15-watt tube is about 18 inches

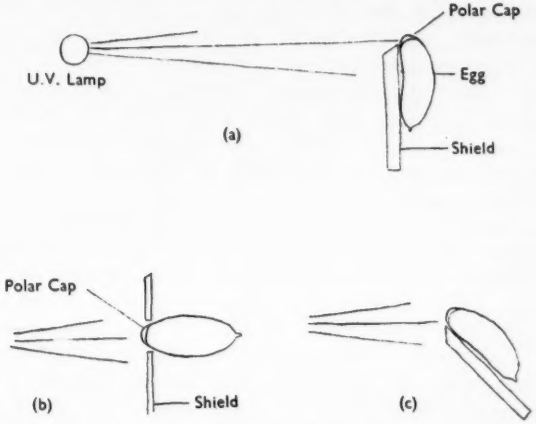

FIG. 2.—Methods of treating the polar cap. (*a*) From the side (rays that strike polar cap are at right angles to long axis of egg), (*b*) from above (rays parallel to long axis of egg), (*c*) rays at 45° to long axis of egg (and therefore at about 60° to plane of polar cap). In (*a*) and (*c*) the egg is resting on its dorsal surface on the shield. In (*b*) the dorsal surface happens to be parallel to the plane of the page, and the egg is supported only in the hole of the shield.

long and ¾ inch wide. Therefore the intensity of the light received by the eggs is not exactly proportional to the inverse square of the distance of the eggs from the lamp, as it would be for a point source. However, the proper correction was made for this fact in calculating the relative intensities of the light received by the eggs at the different distances from the lamp, in the work reported by the Rice Institute group, while in that of the Indiana University group the intensities were measured by a photometer.*

For the purposes of the present experiment it seemed best in a part of the work to vary the dosage of the ultraviolet light by varying the intensity rather than the duration of the treatment. For if the effect of a hit is short-lived, then this effect

* The authors are indebted to Dr J. E. Evans, of the Physics Department of The Rice Institute at the time this work was done (and now at Los Alamos), for help in making the calculations referred to.

might not last from one interval or subdivision of the treatment to the next, so that the addition of a second equal interval would add mainly those mutations which were produced entirely within this interval. That is, if two or more hits were necessary for a mutation, many of those produced in the first interval would be unable to cooperate with those produced in the second interval, so as to cause a mutation. On the other hand, if the dosage is increased by increasing the intensity of the ultraviolet light (and the time kept constant), then as the dose is raised the chances of a coincidence of two or more hits within a given interval are increased logarithmically. Much of the ultraviolet work involving rate-dosage relationships which was done before that herein reported is open to criticism because only duration, and not intensity, of the radiation was used in variation of the dosage. Experiments in which the duration is varied are also useful, however, when comparison of their results can be made with those from the experiments in which the intensity is varied.

3. PRELIMINARY EXPERIMENTS AND GENETIC TECHNIQUES

An extensive series of preliminary experiments was run to determine the maximum dosage of ultraviolet tolerated by eggs in the polar cap stage. But the results of these experiments were rather indefinite, because the dose tolerated varied from one lot of eggs to another. Moreover, the eggs are subject to damage in being shelled and otherwise handled, and in particular they are unduly subject to drying out after being shelled. The damage thus done may vary from one day to another, depending on personal factors, and this influences the percent of eggs that survive a given treatment. In the later experiments, increased proficiency in the handling of the eggs decreased the damage due to the handling. The doses finally selected for treatment in the earlier experiments proper were those that as a rule did not kill or sterilise over about a third of the eggs in most of the preliminary experiments. But in the later experiments higher doses were employed. In some of these the percent of eggs that survived handling and treatment and that developed into fertile males was rather small (less than 2 per cent.). As the shelling and accurate lining-up of the eggs is an arduous process and has to be done in the limited interval of about one hour between the collection of the eggs and expiration of the polar cap stage, the number of eggs successfully treated and bred in any one experiment was rather limited.

In the earlier experiments lethals in the *X* chromosomes were looked for, by means of a recent genetic technique and stocks developed by Muller, but it turned out that the induced rates were so low that no conclusions could be drawn from these experiments. The low recovered rates may have been due in part to the relatively low dosages of ultraviolet employed.

It was then decided to look for lethals in the second chromosome since this has about twice as much euchromatin as the *X* and therefore would be expected to have about twice as high an induced mutation rate. With X-rays in fact, the rate has been reported to be two and a half times that of the *X*. Moreover, heavier doses of ultraviolet

light were employed to further increase the rate. The lethals were detected by means of Muller's *sifter* technique, which has been described more fully elsewhere (Muller, 1951).

In accordance with that form of the *sifter* technique which was used in most of the work done with it at the Rice Institute, males of second chromosome composition *cn bw/cn bw sp* are treated in the polar cap stage of development (*cn* = cinnabar, *bw* = brown, *sp* = speck). These males are mated to females which contain Curly cinnabar^2 and speck^2(*Cy, cn*^{2} *sp*^{2}) in one second chromosome and morula blistered (*mr bs*) in the other, thus *cn bw sp/cn bw* ♂ × *Cy cn*^{2} *sp*^{2}*/mr bs* ♀. This is termed the P_1 cross. The Curly cinnabar offspring (F_1) are of two classes (1) *cn bw sp/ Cy cn*^{2} *sp*^{2} (speck) and (2) *cn bw/Cy cn*^{2} *sp*^{2} (non-speck). About 10 F_1 males of each class, or some other convenient number derived from each P_1 male, are selected as P_2 and mated to females of *sifter* stock. The *sifter* stock is a translocation heterozygote for two *œnothera*-like complexes involving rearrangements of the second and third chromosomes. One of these complexes (the *Cy complex*) contains, among other things, Curly with its left and right inversions and the Pale insertion (P^i). The other contains the Pale deletion (P^-). The stock is viable because P^i in the one complex compensates for P^- in the other. In outcrosses, however, the only viable offspring are those that receive the *Cy* complex. Those that receive the P^- die because they do not receive the compensating P^i. To summarise :

P_1 *cn bw sp/cn bw* ♂ × *Cy cn*^{2} *sp*^{2}*/mr bs* ♀ (♂ irradiated in polar cap stage)

P_2 10 brother F_1 *cn bw sp/Cy cn*^{2} *sp*^{2} ♂♂ and 10 brother F_1 *cn bw/Cy cn*^{2} *sp*^{2} ♂♂ from each P_1 ♂, individually bred × *sifter* ♀♀ (having "*sifter Cy*" chromosome)

P_3 *cn bw sp/sifter Cy* ♂♂ and ♀♀ and *cn bw/sifter Cy* ♂♂ and ♀♀ (10 mass cultures of each kind, one from each of the 10 brother F_1)

F_3 Look for homozygotes (*cn bw sp/cn bw sp* and *cn bw/cn bw*).

The *cn bw* combination of the above homozygotes, when uncomplicated, produces white eyes, in conspicuous contrast to the rather bright red eyes of the *Cy* heterozygotes, so that a nonlethal culture (one containing homozygotes) can be identified at a glance by observing a few white-eyed flies through the glass container. However, in a considerable fraction (about two-thirds) of the homozygotes the expression of *bw* is suppressed by the normal allele of *bw* present in a chromosome section (the Pale insertion, P^i) which was derived from the P_2 *sifter* parent ; hence the eyes of these flies appear cinnabar (bright red) instead of white. However, all the homozygotes, whether white or cinnabar, are recognisable by their straight (non-*Cy*) wings. A nonlethal culture might therefore be identified by means of a few straight cinnabars, in case it happened to contain no

whites, although ordinarily the nonlethal cultures are most readily identified by means of the whites.

It might sometimes happen by accident that a P_1 female (a Curly) was non-virgin. The F_1, F_2 and F_3 derived from her would in that case fail to have a chromosome of treated origin. They would, however, appear Curly in the F_3. *A* P_3-F_3 culture might therefore appear lethal, simply because it did not contain the *cn bw* (*sp*) chromosome. In order to have a check on such accidents, some of the Curly F_3 offspring were always crossed to *cn bw* stock to be sure that they contained the *cn bw* chromosome before the culture was scored as a lethal.

All " brother " lethal cultures, *i.e.* those derived from the same kind of second chromosome (*cn bw* or *cn bw sp*) of the same treated P_1 male, are tested for allelism, in order to determine whether the series represents just one lethal, or two or more of independent origin. This is done by crossing virgins from one lethal culture to males of each of the others. Any cross which produces no whites is considered one in which both brother cultures contain the same lethal. If now the entire series of crosses between brother cultures produces no whites, then all members of the series, being allelic to the same lethal, are considered as allelic to each other and derived from the same mutant polar cap cell. On the other hand, if a cross between two members of the series produces whites, then the parents contain lethals at different loci and hence are of independent origin (having arisen in different cells of the polar cap of the treated male). When more than one lethal non-allelic to that in the culture providing the virgin females are found, they are tested similarly for allelism with each other.

In the *sifter* stock there is some crossing over between the left and right *Cy* inversion. Though this is rare, it occasionally leads to the loss of *Cy* and the left *Cy* inversion from the *sifter* chromosome. Crossing over would therefore not be suppressed in the left arm of the second chromosome of any females that happened to get this chromosome and one of treated origin in the F_2 or later generations. As a result, such females might produce white offspring, even though the chromosome of treated origin originally contained a lethal. Thus a culture that was really lethal might occasionally be scored as non-lethal. The *sifter* stock as used in later experiments was somewhat modified to take care of this situation by the introduction of additional lethal markers, as explained in the separate paper on the *sifter* technique.

4. SOURCES OF ERROR IN THE SCORING OF LETHALS

It is evident that if there is a pre-existing lethal (one present at or before the time of fertilisation) in either second chromosome of a P_1 male (*cn bw sp*/*cn bw*) then that lethal will be contained in the given (*cn bw* or *cn bw sp*) chromosome of all the F_1 " brother " males,

and not just in some or one of the males, as happens when the mutation to a lethal gene takes place in one of the several pole cells of the P_1 male. Hence, when the same lethal was found in an entire series, or " complete run ", as it was called, of " brother " P_3-F_3 cultures (all derived from the same kind of chromosome, or genome, of a given P_1 male), it was regarded as a " pre-existing lethal ", one present before treatment (probably in a parent of a P_1 male), and was not counted as having been induced. An occasional induced lethal is thereby lost sight of, however. This happens when the mutation occurred in a pole cell which managed to give rise to all the offspring (F_1) that were bred to test the given genome—an event that may have been caused by destruction of all the other germ cells by the ultraviolet, or merely by the disproportionately great amount of multiplication of the given cell.

A mutation was classed as a lethal or sublethal, in the Rice Institute experiments, when flies homozygous for the *cn bw* (*sp*) chromosome containing it had a viability of less than about one-thirtieth the viability of their heterozygous Curly sibs, thus changing the ratio of the former to the latter from the expected 1 : 2 to less than 1 : 60. Now the P_3-F_3 cultures in that work usually contained, when counted, some 20 to 30 flies, of which number some 7 to 9 homozygous *cn bw* (*sp*) (either with or without the Pale insertion, P^i) were to be expected, in the absence of a mutation in the *cn bw* (*sp*) chromosome. In case there was a sublethal mutation such that, on the average, only one out of every 30 expected *cn bw* (*sp*) developed, the homozygous type would as a rule not make its appearance at all in a given culture, and so the culture would be tentatively classed as a suspected lethal, tested further, and finally scored in the combined lethal-sublethal category. But, if a homozygote did happen to appear in such a culture, particularly in one containing few other flies, and if this were the first sublethal culture of a series of brother cultures examined, so that the investigator was not forewarned that it might be a sublethal, the culture might inadvertently be scored as nonmutant and discarded. This error would be unlikely, however, where there were sublethal brother cultures and where, as in the later work, all brother cultures were kept together in a group in the same portion of the box of cultures, to be recognised as such and scored in close succession.

Insofar as this source of error existed it would tend to result in a lower recorded mutation rate than the actual one, except in cases in which all of a group of brother cultures had the same sublethal, derived from a pre-existing mutation, but in which one or more of them, that had been spatially separated from the rest, had been erroneously scored as nonmutant with the result that the rest might be considered as of later origin and therefore induced. As pre-existing lethals would appear as often in the controls these would furnish a measure of the frequency of such errors ; they turned out to be few. Nevertheless, further to guard against them, for experiments in which

the brother cultures had not consistently been kept in groups, two methods of calculation were employed in those series in calculating the mutation rate, and both results are presented. According to one of these methods (*a*) the mutations in question—those which had been recorded as appearing in a large but not entire series of brother cultures (*i.e.* the large but not " complete runs ")—were counted as induced, while according to the other method (*b*) they were classed as pre-existing sublethals and therefore not counted as having arisen in the experiment proper.

In the work done at Indiana University the brother cultures were always kept in groups, so that the given error could hardly arise unless a culture had been wrongly grouped. In the Indiana University work, however, the dividing line for classifying cultures as sublethal or nonlethal was at one-tenth instead of one-thirtieth of the expected number of homozygotes. Here it was the practice to retest, by breeding another generation, not only apparently lethal cultures but also those giving unusually low frequencies of homozygotes. The difference in the position of the dividing lines used by the two groups of investigators would not be the cause of much difference in the results, because tests have shown that only a comparatively small proportion of suspected lethals actually falls within the range between these two lines.

Somewhat more important, in the work at both places, was the error caused by the fact that in some cases a series of brother P_3-F_3 cultures was very small, with just a few cultures that proved fertile instead of the desired 10 (or more) derived from the same treated chromosome. In such cases, when all the cultures of a genomal sibship contained the same lethal, it was much less certain that the mutation was a pre-existing one. Had the series been larger, a nonlethal culture might have been found in some of these cases and this would have proved that the mutation should have been counted as having occurred after treatment. We have therefore followed the practice of not counting any all-lethal sibships that consisted of fewer than 5 (or, in some Rice Institute series, fewer than 4) fertile cultures. This has made it necessary to reject, in addition, all other series of fewer than 5 fertile cultures, whether or not they contained any mutations ; for if we had included these and rejected the all-lethal sibships we would have created a bias in favour of a lower lethal mutation rate.

5. STATISTICAL TECHNIQUE OCCASIONED BY MUTATIONAL CLUSTERS

The fact that many of the induced mutations occurred in clusters necessitated the development of a new statistical method for the calculation of the error of sampling. This allows for the fact that each such cluster must be treated as a single event, with a correspondingly high error, in relation to its effect on mutation frequency,

even though in the calculation of that frequency each mutant F_1 must be counted separately, for obtaining the sum of the mutants divided by the sum of mutants plus nonmutants. The formula for the error of the mutation frequency so obtained, worked out by Muller (1952) * is as follows :

$$e_p = \frac{\sqrt{q \cdot \Sigma(c_m \cdot m^2)}}{n}.$$

In this formula e_p is the standard error of p, the observed mutation frequency based on all the given material ; q is $1-p$; n is the number of chromosomes tested ; m is the number of mutants of common origin found in any given F_1 sibship (*i.e.* the size of the cluster) ; and c_m is the number of clusters of the given size (m), found in all the material. In other words, in order to get the standard error by the above formula we square all of the lethal clusters (m^2), get their sum, *i.e.* $\Sigma(c_m \cdot m^2)$, multiply this sum by the percent of nonlethals (q), take the square root of this product, and divide it by the total number of chromosomes tested (n).

This formula is still defective in assuming that there is as much variation in mutation frequency (apart from that caused by clustering) between F_1 derived from different pole cells of the same P_1 cap as between those derived from different P_1 caps, so that the actual error of sampling would be slightly larger than that reckoned. However, results recently obtained by Meyer and Muller, and not included in this report, indicate that polar caps of the same dosage series differ little from one another in regard to the amount of ultraviolet they receive (or in their sensitivity to it), since those caps in which mutations had been produced in the second chromosome were not appreciably more likely than the others to have had mutations in their third chromosome. This indicates the substantial correctness of the error given by the above formula.

6. RESULTS

Table 2 gives the results of two series of experiments, carried out at the Rice Institute laboratory, in which second chromosome lethals were looked for by the *sifter* method above described. The polar caps were irradiated from the side (at an angle of about 15° to the plane of the polar cap) and only the intensity of the radiation in each series was varied, not the time, one series receiving a treatment of 4 minutes, the other one of 8 minutes. In each series, two lots of eggs were irradiated, one lot at 25 cm. from the lamp, the other at 45 cm., the intensity, and therefore the dose, at the near distance being about 2·9 times that at the far.

* In that article (an abstract which explained how the formula was arrived at), the symbol r was used in place of m, I_r in place of c_m, and s_p in place of e_p. The value there given was that of the square of the error instead of the error itself, but by a typographical error n in the denominator was represented without the exponent 2.

TABLE 2

Series designation	Time exposed (min.)	Distance (cm.)	Relative intensity	Relative dose*	No. of chrom. tested	Av. no. chrom. tested per P_1 genome	Per cent. new lethals (incl. large "runs")	Stand. error, this per cent. new lethals	Calc. per cent. induced lethals with st. error	Relat. freq. of ind. lethals*	Per cent. new lethals (excl. large "runs")	Stand. error, this per cent. new lethals	Calc. per cent. induced lethals with st. error	Rel.* freq. of ind. lethals
1A	4	45	1·0	1·0	1091	7·9	9·0	2·0	6·8±2·1	1·00	7·7	1·8	5·5±1·9	1·00
2A	4	25	2·9	2·9	914	8·5	12·6	2·9	10·4±2·9	1·53	11·3	2·6	9·1±2·7	1·66
3A	0	...	0	0	650	6·4	2·2	0·6	0	0	2·2	0·6	0	0
4B	8	45	1·0	1·0	372	6·2	9·9	3·3	9·1±3·4	1·00	6·7	2·3	5·9±2·4	1·00
5B	8	25	2·9	2·9	199	5·9	13·6	6·8	12·8±6·9	1·39	10·9	5·0	10·1±5·1	1·70
6B	0	...	0	0	185	7·1	0·8	0·8	0	0	0·8	0·8	0	0

* The "relative doses" and the "relative frequencies of induced lethals" are expressed as multiples of the doses and frequencies obtaining at the lower dosage intensity, in the series of the same letter (same dosage time), taking the values at the lower intensity as 1. The series designated with the letter A were done at a different time from those designated B. It is for this reason that they have separate controls (3A and 6B, respectively) and that, being not strictly comparable, the relative doses and frequencies for the two groups have not been expressed in terms having a common standard of reference. In all cases the eggs were irradiated from the side (at approximately 90° to the egg axis).

If, for a first consideration, complete runs are excluded but all incomplete runs, even the large ones, are included in the calculation of mutation rates, then in the case of the series given the 4-minute treatment the rate for the near distance proves to be only one and a half times that for the far distance, although the former dose was nearly three (2·9) times the latter. Similarly, for the 8-minute treatment, the rate at the near distance was 1·4 times that at the far, despite the 2·9-fold greater dose of the former. Thus the ultraviolet at the higher dose was only half as efficient in giving rise to observed lethals. When large but incomplete runs are excluded from the calculation, on the assumption that they represent pre-existing sublethals, the relationships are not significantly changed in either the 4-minute or 8-minute treatments, although in both cases the apparent fall in efficiency is somewhat less marked. The latter feature of the results would indicate that the large but apparently incomplete runs probably were, in the main, pre-existing, since of course the pre-existing lethals would not tend to rise at all with dose, but the absence of such cases from the controls would argue for these lethals having been induced. Even with these large runs excluded, when both the series at 4 and 8 minutes are considered together, the fall in mutagenic efficiency with dose turns out to be significant, if the calculation of significance is based on the errors obtained by the formula previously presented.

If now, in table 2, the results from flies treated with different durations of ultraviolet, but with what was intended to be the same intensity, are compared, it is found, similarly, that doubling the time increased the mutation frequency by a much smaller factor than 2 (by only about 1·1, when the lethals of large runs are assumed to be pre-existing). This conclusion is reached by comparing the results of the upper line of the 4-minute treatment with the upper line of the 8-minute treatment, and the lower line of the former with the lower line of the latter. Thus, whichever way the dose was increased, the increase failed to produce anything like a proportionate rise in mutation frequency, at these dosage levels. The comparison of the effect of different durations is not nearly as reliable as that of different intensities in this case, however, since the lots differing in duration were done at different times of the year, and not so strictly parallel in other respects.

Table 3 shows the results of several series of treatments carried on at the Indiana University laboratory in the summer (S) and fall (F) of 1948. In this work, as previously mentioned, the ultraviolet formed an angle of 45° with the longitudinal axis of the egg and of some 60° with the plane of the polar cap. At this higher angle much less ultraviolet was required for attaining the same mutation rate than when it shone from the side ; this is a main reason why the longer distances here used gave higher rates than those shown in table 2. Cases of complete runs, signifying pre-existing lethals, are

TABLE 3

Series designation*	Time exposed (min.)	Distance (cm.)	Relative intensity†	Relative dose‡	No. of chrom. tested	Av. no. chrom. tested per P_1 genome	Per cent. of tested eggs forming larvæ	Per cent. of larvæ forming adults	Per cent. of adults fertile	Size of "run" (av. per cent.)	Per cent. of new lethals found, with st. error	Calc. per cent. induced lethals, with st. error
1 (S)	6	150	1·8	3·6	337	8·9	55·4	37·1	81·2	23·3	17·2±4·1	16·6±4·1
2 (S)	6	100	4	8	335	8·8	43·1	27·9	42·3	29·4	20·0±4·4	19·4±4·4
3 (S)	3	100	4	4	354	10·1	41·8	58·8	70·3	22·5	15·0±3·5	14·4±3·5
4 (S)	3	50	14	14	183	9·6	28·9	20·7	42·5	33·0	18·0±5·8	17·4±5·8
5 (S)	0	...	0	0	1387	9·1	54·0§	85·3	95·4	16·6	0·6±0·4	0
6 (F)	3	200	1	1	807	9·6	76·4	80·0	92·1	22·4	12·0±2·0	11·1±2·0
7 (F)	3	100	4	4	543	9·4	62·0	57·4	79·4	25·8	19·1±3·2	18·2±3·2
8 (F)	10½	200	1	3·5	427	9·5	61·3	62·4	82·4	24·6	21·8±3·9	20·9±3·9
9 (F)	0	...	0	0	639	9·5	47·2§	78·5	95·6	11·0	0·9±0·4	0

* (S) refers to series of experiments carried out in summer, (F) to those in fall, of 1948, at Indiana University. Incident ultraviolet formed an angle of 45° to the egg axis.

† 1 unit of relative intensity represents 0·61 ergs/sec./mm².

‡ 1 unit of relative dose represents 111 ergs/mm².

§ These controls were defective in so far as survival of the eggs was concerned since they were subject to too much heat and drying after dechorionation. This happened because the handling of the control eggs was here postponed until all the treated ones had been attended to. Such differences in handling also affect the other figures in this column to some extent. However, they have very little effect on the results in the next two columns which therefore afford a more reliable index of the physiological effects of ultraviolet.

excluded from table 3. However, long runs, when incomplete, are included, since in all this work special procedures were followed to avoid recording a sublethal culture as nonlethal. These procedures included the grouping of brother P_3-F_3 cultures together in the container and the testing of those cultures which in F_3 had given a low number of homozygotes, until at least 100 flies of F_3 type had been obtained, the upper limit of viability for homozygous sublethals here being taken as 10 per cent. of the viability of the Curly heterozygotes.

The data have been grouped so as to show in juxtaposition those which involved treatments of the same duration but different intensity. It will be seen that the results of changing the dose by changing the intensity, as recorded here, are entirely in accordance with those of table 2, which were obtained independently by the Rice Institute workers during the same year. In the case in which the dose was a little more than doubled by changing the intensity (lines 1 and 2) the induced mutation rate (after the control value has been subtracted) was raised by only about 17 per cent. of its lower value, while in the cases in which it was nearly quadrupled (lines 3 and 4) or quadrupled (lines 6 and 7 and lines 6 and 3) the rate was raised by only between 22 and 64 per cent.

It is also to be observed that when the dose was doubled by increasing the time only (lines 3 and 2) the mutation rate was raised by only about 35 per cent., and when it was nearly quadrupled by raising the time (lines 6 and 8) the rate was raised by only about 87 per cent. It should be mentioned in this connection that in the work reported in table 3, unlike that in table 2, lots differing in duration of treatment were (when of the same series, S or F) irradiated on the samc occasion, just as were those differing in intensity, and that they therefore present as valid a parallel with one another, for comparison of the results of altering the dose, as do the series differing in intensity.

Although, on account of the relatively large sampling errors, we remain very uncertain as to the precise relations, nevertheless it is clear that a rise in the dose, no matter whether achieved by an increase in intensity or time, brings about nothing like a proportionate increase in mutation rate. Another way of expressing this relation is to say that, at low levels of dosage, a given rise in dose must produce a greater increase of mutation frequency than at most of the levels used in these experiments, *i.e.* the curve expressing the relation of dosage, as abscissa, to mutation rate, as ordinate, must on the average have gone up more steeply between its origin and the lowest dose here used than after that point. Study of table 3 also suggests that, between the different doses here studied, the curve continues to become more nearly horizontal, until possibly it may even decline somewhat.

In the same experiments counts were made of the percentage of treated embryos which hatched to form larvæ and of the percentage

of these larvæ which survived to the imaginal stage. It will be seen that survival at both stages is affected markedly by dose, although later studies have indicated larval survival to be a more reliable guide to dose than the egg hatching rate. At the higher doses, even though the mutation rate shows little if any increase, the survival rates are, if anything, more affected by a given factorial rise than at lower doses. The fertility rate is seen to vary similarly to the survival rate.

We have listed in addition the average size of cluster or length of " run ", calculated as explained on p. 155. The trend of these figures shows clearly the influence of higher doses in increasing the size of cluster. This is an expression of the reduction, by the ultraviolet, of the number of pole cells which proliferate to give rise to gametes. This index, like larval survival, seems to have the curve of its rise less subject to flattening out, at higher doses, than that for mutation rate.

TABLE 4

Irradiation parallel to egg axis

Series designation	Time exposed (min.)	Distance (cm.)	Relative intensity	Relative dose	No. of chrom. tested	Av. no. chrom. tested per P_1 genome	Per cent. new lethals with stand. error	Calc. per cent. induced lethals with st. error	Relat. freq. of ind. lethals
1*P*	½	45	1·0	1·0	783	16·3	4·2±1·4	3·4±1·5	1·0
2*P*	½	25	2·9	2·9	889	22·2	9·0±2·4	8·2±2·5	2·4
3*P*	0	...	0	0	629	13·1	0·8±0·5	0	0

Table 4 gives the results of a later series of experiments, carried out at the Rice Institute laboratory, in which the polar cap was treated from above. Here the incidence of the ultraviolet to the egg axis was 0° and to the plane of the polar cap about 75°. In this table, as in table 3, the more nearly vertical illumination has resulted in a higher mutation rate, for a given exposure, than that, shown in table 2, which resulted from a side illumination. Thus, according to table 4, a ½-minute treatment from above at the 25 cm. distance gave a mutation rate of 8 per cent., almost as high as the rate of 10 per cent. which according to table 2 was given by a 4-minute treatment from the side at the same distance. Moreover, one or more recovered lethals were induced in as many as 17 out of 20 treated polar caps when the treatment was from above, as compared with a proportion of only 37 out of 54 when treatment was from the side. Yet despite the relatively high mutation rate the number

of lethals in a run was smaller from polar caps treated from above than from the side. This effect, which had the advantage of reducing the error of sampling, was doubtless caused by the greater evenness of the illumination from above. For with this more even illumination fewer cells would be so heavily exposed as to be inactivated, while those not inactivated would nevertheless receive more ultraviolet than if they had been shaded by the others.

In table 4, as in 1, 2 and 3, there appears to be a falling off of the mutation rate relative to the dose, as the dose is increased, for the higher dose is 2·9 times that of the lower, while the mutation rate at the higher dose is only about 2·4 times that at the lower. Inspection shows, however, that the difference between these ratios is less than its own standard error, and it is also evident that the approach to a linear relation is significantly closer than in any of the previous experiments. The closer approach to linearity in this series of experiments is to have been expected on two grounds. First, a lower range of dosage, giving a lower range of mutation rates, was here being used. That is, the more steeply rising portion of the mutation rate-dosage curve was dealt with. Second, the more vertically impinging ultraviolet here used, with its more even distribution, minimises the selective effect that tends to reduce the frequency with which lethals are recovered at high doses (see discussion in the following section).

7. INTERPRETATIONS

The results of the present experiments show that the frequency of recovered mutations induced by ultraviolet light does not increase proportionately with rise in dose, for the doses used by us, when the dose is varied by changing either the intensity or the time. That is to say, the induced rate found, per unit dose of ultraviolet, decreases with increase in dose, when either the time or the intensity is held constant. Thus, the rate-dosage curve (with rate as the ordinate) at first rises and then tends to level off until it reaches a point where further increase in dose causes no further appreciable increase in rate.

The results thus agree, so far as they go, with those first obtained by Hollaender and his co-workers (1939 *et seq.*) for visible mutations in fungi of various kinds after exposure of the spores to ultraviolet, and by Stadler and his co-workers (1939 *et seq.*) for endosperm deficiencies caused by chromosome breakage after ultraviolet treatment of maize pollen. However, we have not in the present experiments found a significant drop in the rate-dosage curve at the highest doses. This was first definitely found in the work with the fungi, and was later found by Sell-Beleites and Catsch (1942) for lethals in *Drosophila* spermatozoa which were exposed to ultraviolet through the body wall of the male.

The results obtained in *Trichophyton* (Hollaender and Emmons, 1941) and later in some other fungi show that the decrease in mutagenic effectiveness of ultraviolet with rise in dose is caused at least in part

by its physiologically damaging effects being exerted with greater force, at higher doses, against those cells which have had mutations induced in them, as compared with nonmutant cells, on account of the lower viability of the mutants. In other words, the physiological damage acts synergistically with the genetic damage, in reducing viability. The existence of this selective effect was shown by the finding that post-irradiation treatments which favoured recuperation of the fungus spores from the ultraviolet allowed relatively more of the mutant cells, as compared with the nonmutants, to be recovered. At very high doses the selection against the mutants may even become so strong that the surviving cells include a smaller percentage of mutants than at lower doses. Further evidence for these conclusions is afforded by the finding by Swanson, McElroy and Miller (1949) that when spores of *Neurospora* are treated with ultraviolet the mutation rate-dosage curve of the morphological mutants shows a levelling off and drop with increase in the dose, while that of the biochemically deficient mutants fails to do so, inasmuch as the latter mutants, unlike the former, are protected, by the complete nutrient medium used, from the detrimental effects on survival which their abnormalities would otherwise have occasioned in them.

In our *Drosophila* material, a similar protective action against the effects of the mutations on the cells is, in the case of autosomal genes, exerted by the presence of the unmutated allele in the homologous chromosome. As most of the gene mutations we have dealt with are, except to a slight degree, recessive, even in their action on the individual as a whole, and would usually have less effect still on the life of mutant cells surrounded by a nonmutant body, there could have been very little direct selective effect of the ultraviolet against cells with autosomal mutations of the sort studied by us. This possibility then can be ruled out as a direct explanation of the marked levelling out of the curve of autosomal mutation frequency shown in our data.

It was recognised by Hollaender (1939) that inequalities in ultraviolet illumination could also lead to a flattening of the mutation rate-dosage curve, by killing off, at higher doses, a larger proportion of the spores which had been more exposed. However, he considered it unlikely for this situation to hold for the minute spores of *Trichophyton*. Stadler, on the other hand, noted that among his maize pollen there would be marked inequalities in illumination of the chromosomes of different spores, due to the eccentric positions of their nuclei, and he calculated that the resulting selective effect was sufficient to account for the convexity of the rate-dosage curve. Later, evidence was adduced by Hollaender, Sansome, Zimmer and Demerec (1945) that there were inequalities in illumination and/or " response " to illumination among *Neurospora* spores. In any situation involving uneven illumination, when the dose rises high enough to kill off or retard the multiplication of some of the cells, the frequency of recovered (*i.e.* found) mutants will be lower than that of actually arising mutants.

For the more illuminated cells, in which there is a higher mutation rate, will have been selectively killed off or retarded in multiplication. And the higher the dose rises the stronger will this effect be, so that the recovered rate will become lower and lower in proportion to the actual rate, with progressive flattening of the rate-dosage curve.

Differences in illumination alone would not cause an actual drop in the curve at high doses, however, unless the cells were in two or more rather discontinuous groups, in respect to the amount of illumination received by them, with comparatively few cells receiving intermediate amounts. That is, the frequency distribution of illumination would have to follow a bimodal or polymodal rather than a unimodal curve or a straight line. For if the curve were not bi- or poly-modal, then as the dose was raised and selection killed off the cells with the highest mutation rate, those in the next lower category of illumination would now be getting as much light as the most highly illuminated had at the previous dose and their mutation rate would be as great. As this would apply to the cells all along the line the over-all recovered mutation rate would actually be slightly higher, with any rise in dose, since with each increment the cells in even the very lowest category of illumination would have moved up. On the other hand, with a polymodal curve or—what amounts to the same thing—a fairly sharp discontinuity in the amount of illumination received by different groups of cells, there could be a drop in rate at higher doses. For in that event when the dose was raised, the most affected group might be virtually eliminated at a dose at which there was not yet sufficient illumination of the remainder to bring their mutation rate up to the previously existing average rate. It is to be noted, however, that a rise in dose beyond this would again bring the rate up, supposing that it was practicable to increase the dose further. Thus, there would tend to be a rate-dosage curve the modes of which corresponded with those of the illumination-categories. Such findings have not yet been reported.

There is no doubt that in our *Drosophila* material there must be some inequalities in the amounts of illumination received by different polar caps. For it is impossible to place them all in just the same position relative to the source of light or to shade them in precisely the same way. They also differ somewhat from one another in regard to their stage of development and the number, size, shape, arrangement and opacity of their contained cells and cell parts. All these differences are probably very minor, however, compared with those differentiating the cells of the same polar cap. Certainly, with side illumination, the far cells must be much more shaded than those nearest the source of light. But even with vertical or 75° illumination, although the cells in the same layer can shade each other very little, those on the surface must receive much more ultraviolet than the underlying ones.

It can be calculated from measurements of protoplasmic penetrability which have been reported for other material (see Lea, 1946)

that light of the wave length here used by us (2537 Å) might be reduced to as little as about a tenth its intensity by passing through a cell the size of a pole cell (about 5 micra in diameter). A slightly different absorption coefficient (such as the present material might have) could change this figure considerably. Now a reduction to about 1/18 the intensity would suffice to explain our finding that a 14-fold rise in dose occasioned a rise of only about 50 per cent. in the mutation rate. This can be reckoned as follows. With a difference of 18 times between the illumination of the layers, if we assumed that at the 14-fold dose *all* the top-layer cells had been killed, the lower-layer cells now remaining would be getting 14/18, or 0·78 times, as much ultraviolet as the top-layer cells received at the lesser dose. Since at the lesser dose the lower cells would be getting 1/18 as much as the upper cells, the average illumination of both layers at this dose would be $(1/18+1) \div 2$, or 0·53, of the amount received by the top-layer cells alone at the lesser dose. Therefore the figure 0·78/0·53, or 1·5, would represent the ratio between the amounts of ultraviolet received by the actually functional germ cells of the polar caps at the greater as compared with the lesser doses. It is further to be observed that since the cells are to a large extent arranged in two layers (with perhaps an occasional cell still lower down), even that condition of comparative discontinuity of grouping is provided which might lead to a drop of the rate with increase in dose.

That in our material ultraviolet does have a damaging effect, varying with dose, on cell multiplication and/or survival is evident from the figures we have given on the dose-dependent lowering of the hatchability of eggs, viability of larvæ and fertility of the imagos which emerge. Moreover we have found (observations of Meyer) a pronounced delay in development, of both embryonic and larval stages, which also varies with dose. There is little reason to doubt that such effects would likewise differentiate cells of the same embryo which had been illuminated to different extents. That the different pole cells of the same cap are subject to different degrees of damage has been shown by our finding of the increase in the relative size of mutant clusters (*i.e.* of the reduction in the number of germinally functional germ cells) with rise in dose.

The fact that inequality of illumination of different cells of the same cap is an important factor in determining which cells are to be inactivated (*i.e.* that the damage is selective) is shown by the larger cluster size, implying a larger proportion of pole cells inactivated, in connection with the production of a given mutation rate, when the illumination is from the side than when it is more nearly vertical. It is shown, secondly, by the fact that a larger total dose is needed to attain a given frequency of recovered mutants with side illumination as compared with more vertical illumination. And it is shown, thirdly, by the fact that, within dosage ranges of not very different mutagenic effectiveness for both kinds of treatment, vertical illumination allows

the mutation frequency to rise more nearly in proportion to increase in the dose than does side illumination. The conclusion is thereby indicated that in our material the inequality in the amount of ultraviolet reaching different cells, through its selective action against the survival of the more illuminated ones, is the chief factor, or at least a major factor, responsible for the falling off of the mutation rate relative to dose at comparatively high doses.

It is evident that if cells of the same or of different polar caps should differ from one another in their sensitivity to the action of ultraviolet in retarding their multiplication and in killing them, and if these differences were positively correlated with differences in their sensitivity to the mutagenic action of ultraviolet, we should have a situation the effect of which would be like that of differential ultraviolet illumination of the cells. Like the latter influence, therefore, it would lead to a levelling off of the mutation rate-dosage curve with rise in dose, and could also lead to one or more drops in the curve, if the cells were grouped more or less discontinuously in regard to their sensitivities. It seems unlikely however that the *Drosophila* pole cells, either of the same or different embryos, would differ greatly in their sensitivity to ultraviolet. It is true that at an early period of the polar cap the polar granules within the cells gradually fade and at the same time the nucleus assumes a more typically " resting " configuration. However, the cells change little in appearance thereafter and have evidently settled down into a long-lasting interphase of considerable stability. In this condition we could hardly expect some of them to be some 18 more times as sensitive as others—to correspond with the 18-fold differences in illumination which we found, on p. 172, to be necessary for explaining, on that interpretation, the pronounced levelling off of the curve at high doses. Since, on the other hand, there is reason to believe that such differences in illumination may exist, it is not necessary to suppose that any considerable role is played by differences in sensitivity.

Granted either differences in illumination or sensitivity or both, with resultant selection on the basis of " physiological damage " against the cells in which more mutations had been induced, this effect would be very slightly enhanced by genetic selection against the same cells. For the unevenness in illumination and/or sensitivity would cause the mutations to have a positive correlation with one another in their distribution among the cells. Although mutations in the autosomes, being so nearly recessive, would very seldom have an appreciable effect on cell survival, many of those occurring in the single *X*-chromosome in cells of males would detrimentally affect their survival or proliferation, as studies on X-ray induction of mutations have shown. These inactivations would reduce the frequency of recoverable mutations in the autosomes as well, because of the correlation of mutations with one another. However, it can be calculated that this effect would be of too low an order of magnitude

to account for the levelling off of our curve of second chromosome lethals, or for the reduction in pole-cell number shown by our results on cluster size. For, whereas the latter effects require the elimination of 50 per cent. or more of the pole cells, the induced lethal rate in the *X*-chromosome is far lower than this, and even with a high correlation of mutations with one another there would be a still lower proportion of autosomal lethals thereby eliminated. Hence this selective influence is not comparable in magnitude with that caused by the selective " physiological " (*i.e.* nongenetic) damage attendant upon the unevennesses of illumination.

Ultraviolet is peculiarly subject to selective effects because of (1) its low penetration (high absorption) in cells, coupled with (2) its extremely harmful action on the physiological (including the mitotic) systems of the cell. This applies especially to ultraviolet as short as that used by us. Visible light would be subject to negligible inequalities in distribution within a tissue so thin and transparent as the polar cap, while ordinary X-rays would of course be distributed with virtual uniformity throughout the entire body of so small an organism as an insect.

Selection is not the only mechanism which could result in a tendency of the mutation rate-dosage curve of ultraviolet to level off, and on occasion even to drop, at high doses. The phenomenon commonly referred to as " photoreactivation ", which we now prefer to term " photorepair " (Muller, 1954), could also produce such an effect, provided the material treated with higher doses of the mutagenic ultraviolet also received sufficiently higher doses of reparative light (that which interfered with mutagenesis). This possibility arises from the fact that the amount of repair or interference effected by a given amount of the reparative light is not fixed but is proportional to the amount of damage to be repaired (*i.e.* to the potential mutagenic effect of the mutagenic light). This was shown, for instance, in the experiments of Novick and Szilard (1949) on *E. coli*. Thus if the amount of reparative light rose along with increase of the dose of mutagenic light, a larger and larger *proportion* of the potential mutagenesis would, as it were, become sidetracked, and the slope of the rate-dosage curve would become increasingly depressed (except for the limitation mentioned in the second paragraph below) as the curve proceeded to the right.

It is true that about 90 per cent. of the output of the Germicidal lamp used in our own experiments was of the mutagenic wave length 2537 Å, and part of the rest was also in the mutagenic range. Nevertheless the amount of nonmutagenic reparative light may have been enough to affect our results appreciably, and this amount must have increased along with the rise of dose of the mutagenic light. Filtering had not been resorted to because the experiments were performed prior to the discovery of photorepair as a general phenomenon. Moreover, the possibility cannot be dismissed that even the mutagenic

light itself may in this material have some reparative effect in addition, despite Dulbecco's (1950) finding that this is not true in phage, since the action spectrum for repair is not the same in all organisms (Kelner, 1949, 1952).

Several considerations, however, indicate that most of the decline in slope of our rate-dosage curve at higher doses was not the result of photorepair. One of these considerations concerns itself with the fact that there is a limit to the amount of this effect, set by the circumstance that only a fixed proportion of the potential ultraviolet damage is reparable at all by the reparative light. Suppose for instance that only one-fifth of it was irreparable. Then under no circumstances (provided photorepair was the only factor causing the curve to decline from linearity) would a ten-fold rise in dose produce less than a doubling of the mutation rate, or a fifteen-fold rise in dose less than a tripling of the mutation rate. Thus the fact that in table 2 a fourteen-fold rise in dose produced a mutation rate only one and a half times as great, and that the other data are in harmony with this, indicates that photorepair alone could not have lain at the basis of this effect, inasmuch as in other experiments, in which very strong doses of reparative light were intentionally administered (Meyer, 1951 ; L. and E. Altenburg, 1952), the portion of the mutation rate which remained uninterfered with was about a quarter. A further limitation on the repair effect is that, when the mutagenic and reparative light are increased *pari passu*, a near-maximum level of effectiveness of the latter is after a while attained (provided so high a dose can be tolerated), after which the *proportion* of potential damage repaired remains virtually constant. Thereafter, then, further increases of dose must give the same rate of increase of observed effect as if no reparative light were present at all.

Those features of our data which have already been mentioned as evidences of a selective effect based on inequalities of illumination are not explicable as effects of photorepair. These include the much lower mutagenic efficiency of side as compared with more vertical illumination and the much greater influence of the former type of exposure both in depressing the rate-dosage curve and in increasing cluster size, when the same level of mutagenic effectiveness is maintained in the two lots. Another telling fact in favour of selection is that, at the high doses at which the rate-dosage curve for mutations is practically flat, both the damaging effect on larval survival and the cluster size are continuing to increase with increase of dose. This would not be expected if photorepair lay at the basis of the decline of slope of the rate-dosage curve for mutations since others (first among them, Novick and Szilard in 1949) have reported that photorepair affects survival and mutagenesis in parallel fashion, as though by changing the amount of a substance upon which both depend. Selection, on the contrary, would at higher doses produce an ever more adverse effect on survival, both of larvæ and of individual pole

cells, but by that very means would tend further to reduce the frequency of observed mutants, below the frequency with which they had actually been induced. All in all, then, we are led back to the conclusion that most of the depression of the slope of the mutation rate-dosage curve has been caused by inequalities of illumination.

At the same time, it should be recognised that a part of the explanation of this phenomenon might be even more complicated. The prolonged plateau exhibited by the curve suggests some sort of saturation effect, such as might be caused by the using up of materials which take part in the process of mutagenesis. In bacteria, for instance, as Stone *et al.* have shown, a photon of ultraviolet can often produce a mutation indirectly, through first producing a mutagenic substance in the medium. Now although the correspondence between the absorption spectrum of ultraviolet by nucleic acid and its mutagenic action spectrum in varied plant materials has indicated that most of the mutagenesis in these cases results from quanta absorbed by chromatin itself, the possibility is not thereby excluded that even under these circumstances the process may involve a number of steps. Photorepair, in fact, gives evidence of this. If, now, there were a chain of reactions intervening between quantum absorption and mutation, the probability of occurrence of such a mutation might depend upon the concentration of some substance necessary for the formation of a given one of the links in this chain. The amount of that substance which could be formed might be limited by limitations of the substrate for it, or (what amounts to the same thing) the rate at which it was used up might outrun its rate of formation, with the result that the rate of mutagenesis tended to approach a limit at high doses. Nevertheless, we do not as yet need this additional hypothesis to arrive at a reasonable interpretation of our results.

As the dosage is reduced, both the selective effect and the photorepair, as well as, if it should exist, the effect postulated in the preceding paragraph, must of course become gradually less pronounced, and finally negligible. Only at these low doses could it be determined whether what might be called the primary rate of mutagenesis had a linear relation to ultraviolet dose, as it would have if a mutation was caused by a single "lucky" quantum absorption, or whether several quanta cooperated or interfered with one another. Since our results indicate that, at moderate doses at any rate, most of the hindrance to our observance of the primary rate of mutagenesis is caused by selection, the question arises as to how the dosage level may be recognised at and below which selection plays only a negligible role. The answer, theoretically, is that this must be a level at and below which there is no longer any influence of dose on mutant cluster size. It is however difficult to accumulate a sufficient number of mutations for such a study at such low doses, and the task has not yet been carried through on a sufficient scale to arrive at a definite answer to the question in our material.

It is true that, in such of the work herein reported by us as was done with relatively low doses, administered more nearly vertically, as in table 4, a comparatively linear rate-dose relation was shown. Nevertheless, the selective factor may not yet have been negligible here and it therefore remains possible that the primary rate of mutagenesis varies as a higher power of the dose than one, or follows an even more complicated course. That is, the apparently linear relation may have resulted from our observing a portion of the rate-dose curve which was in the region of transition from a concave to a convex shape. Although the matter cannot be regarded as settled, direct support for this interpretation has very recently been provided by another series of experiments (L. and E. Altenburg and Baker, 1952), involving rather low doses, in which the relative rise in induced mutation rate exceeded, by an amount greater than the sampling error, the relative increase in dose.

In other material, the reported results differ with the organism used. The frequency of chromosome breaks was found by Swanson (1940, 1942) to vary linearly with dose of ultraviolet in *Tradescantia* microspores. Likewise, in the work of Stadler and Uber (1942) on endosperm deficiencies caused by chromosome breaks induced by ultraviolet treatment of maize pollen, it was deduced that the relation would have appeared linear had the nuclei not been eccentric. By analogy, one might expect point mutations also to have a linear rate-dose relation. Such a relation has in fact been found by Kaplan (1948, 1949*a*, *b*) in *Bacterium prodigiosum* for the phenotypically defined mutation to stunted colony induced by ultraviolet.

On the other hand, the results published by Demerec and Laterjet (1946) and by Novick and Szilard (1949) on ultraviolet mutations in *E. coli* give curves the slopes of which would indicate that, over the wide range of dose used by them, two to three hits cooperated in producing a mutation. Similarly, although the earlier results on the frequency of mutations induced by ultraviolet in various fungi appeared capable of interpretation as representing a linear relation at low doses, a definite decision on this point could not be made because of the paucity of the mutations found at such doses, and recent work of Swanson (1952) on *Aspergillus* shows a concavely rising curve, indicative of a multi-hit effect.

Kaplan's further observations on *B. prodigiosum* and on *E. coli* are of special interest in their bearing on the problems here at issue. He found that although the mutation rate-dose relation was linear when ultraviolet was used, it exhibited a two-hit curve when the mutagen was visible light in the presence of erythrosine (Kaplan, 1950*a*). As the rate was at the same time simply proportional to the amount of erythrosine (at low concentrations of the latter) he concluded that a mutation required two quanta of visible light to be accumulated on the same particle of erythrosine but that one quantum of ultraviolet, being larger, sufficed for a mutation

(even without erythrosine, since it could be absorbed directly by chromatin).

On the other hand, Kaplan (1950*b*) found that a special strain of *E. coli*, histidineless, having unusually long cells, showed a multi-hit mutation-rate curve (of back mutations to histidine-independence) even when ultraviolet was used. The latter results are similar to the already mentioned findings of Novick and Szilard on *E. coli*. However, Kaplan found that whereas his long-celled strain also showed a multi-hit survival curve (as had that of Novick and Szilard), another strain (" B ") of *E. coli* gave a one-hit (linear) survival curve with ultraviolet. Although strain B was not tested for mutation rate it seems not unlikely, in view of the difference in survival and in length of cells, that its mutation rate also would have been found to be one-hit. In that case the multi-hit curves might possibly represent a derived condition, caused by a compoundness of the long cells, which allowed only multiple changes to give observable effects.

Although it is hardly possible that a condition similar to the one in the histidineless strain of *E. coli* exists in *Neurospora* or *Drosophila*, it is conceivable that it did in the strain of *E. coli* used by Novick and Szilard. Moreover, it must be conceded that other biological differences might influence a result of this kind. Even more telling is the fact that mutations in the same organism could be produced by either one or two hits, according to the size of the quantum. This indicates that a different rate-dose curve might be obtained by a relatively slight change of wave length, if the transition zone of wave length could be determined. The latter, in turn, is one of the factors which might vary with the biological material. At the same time, the fundamental mechanism of mutagenesis would probably remain much the same in these cases.

8. RECOMMENDATIONS FOR FUTURE WORK ON THE SUBJECT

In view of the various considerations advanced above, taken together with the experience gained and the results here reported, certain specifications for further work on the primary mutation rate-dosage relation in cells of the *Drosophila* polar cap can now be recommended. The work should be conducted, so far as possible, with doses so low as to avoid intercellular selection, as shown by the fact that the size of the " run " has reached its final minimum throughout (with due precautions to avoid a possible false minimum before the final one, as explained on p. 155). In place of light of 2537 Å there should be used the longest mutagenic wave lengths practicable—if possible, those of 2900 to 3100 Å, in order to get a more nearly equal penetration of the ultraviolet among the pole cells in different positions and thus to reduce the selective effect. The illumination should be at an angle of not more than 45° to the egg axis, preferably less,

again in order to give more uniform illumination. To avoid the destruction of too much somatic tissue by penetrating light entering in such a direction, a special shielding method, such as the pinhole technique used by the Rice Institute group, is advisable.

Nonmutagenic light of photoreparative wave lengths, except of an intensity equal to that which is continuously present in the room anyway before and after treatment, should be excluded during treatment, or else should be present in constant amount. One way of meeting this requirement is to vary only the duration, not the intensity, of the treatment with mutagenic light, and to do this removing, for the desired interval, by a filter which rather sharply cuts off the ultraviolet shorter than about 3200 Å but leaves the longer light virtually unchanged ; the exposure to the nonmutagenic light would be equally long and equally intense for all the embryos. Alternatively, a monochromatic beam derived from a spectrum could be employed, but it is less convenient to obtain this at the desired intensity and size of field. There would be an advantage in intentionally exposing all embryos to a considerable and equal dose of nonmutagenic reparative light, since this would tend to swamp out any differences in this respect attendant upon the treatment with mutagenic light. In fact, it would be desirable to use so high a reparative dose as to attain practically maximum repair. For this would even nullify any possible reparative effect of the mutagenic light itself (provided only that the same portion of the damage is reparable by different wave lengths of reparative light, a matter subject to verification). A comparison of the rate-dose curves obtained with and without the nonmutagenic reparative light would in fact disclose whether or not the mutagenic light also acts reparatively.

The attainment of the arrangements above suggested is facilitated by making time, not intensity, the variant in changing the dose. That the effect on mutation rate in the pole cells is the same regardless of which of these two factors is varied has recently been shown, for a nineteen-fold time-intensity range, by E. Altenburg, Bergendahl and L. Altenburg (1952). It might be thought that this result proves mutation to result from a single quantum absorption, but this is not the case. For the finding that both photorepair (Meyer, 1951 ; L. and E. Altenburg, 1952) and thermal repair (Edmondson and Meyer, 1952 ; Meyer and Muller, 1952) of potential mutagenesis can be effected in *Drosophila* pole cells subsequently to the exposure to the mutagenic ultraviolet shows that an interval of the order of several minutes at least (*i.e.* as long as the duration of the treatments themselves) commonly intervenes between quantum absorption and finished mutation. In the meantime there would be opportunity for effects of different quantum absorptions to interact (if that occurs) in the production of a mutation.

A further procedure to be recommended is that, if the males derived from the treated embryos are used for the mutation rate

studies, preference be given to carrying out the tests on the sperm which they release during their first week of imaginal life, since these sperm are derived from a larger number of pole cells than are those released later, and the sampling error is therefore smaller for them. It might be better, however, to have a genetic scheme which would allow the investigation of treated females. For their primordial gonads are composed of approximately twice as many primary germ cells (former pole cells) than those of males, and it is probable that these multiply more equally in giving rise to the later germ cells, since separate egg strings are established rather early. This procedure would allow the testing of a larger number of pole cells per treated individual investigated. Moreover, in females any genetic selection occasioned by correlated mutations in the *X*-chromosome would be minimised. It is true that fewer offspring can be obtained from one female than from one male, but this might be more than compensated for since even the offspring derived from eggs laid in the later weeks of life should be suitable for testing. Unfortunately, however, only one autosome of a pair can by present genetic techniques be investigated in females, since there are pre-existing lethals accompanying the inversions used heterozygously to prevent the production of crossovers.

Chiefly, then, the present experiments must be regarded as doing the heavy work of breaking the ground for more definitive attacks, in showing the nature of the disturbing influences to be encountered, and methods of circumventing them. It is believed that, now this has been done, it should be possible to determine, with a lesser amount of effort than already expended, the primary mutation-rate dosage relation. The choice of this material is justified by the fact that the types of genetic change involved can be determined with comparative exactitude. The problem is one which bears on virtually all fields of radiation genetics since ultraviolet, unlike ionising radiation, gives opportunity for determining the effectiveness of individual excitations.

9. SUMMARY

Studies were made of the rate at which lethals are induced in the second chromosome of *Drosophila melanogaster* by ultraviolet light, predominantly of 2537 Å wave length, applied to the germ cells at the time when they are present in the " polar cap " of the early embryo. Lethals induced at this stage undergo multiplication by cell division and therefore occur in clusters among the gametes. In order to determine the sampling error of the mutation frequency based upon results in which such clusters of mutants of identical origin are present, a new error formula had to be devised. For the detection of the lethals, a new genetic technique, designated as the " *sifter* " method, was employed.

It was found that the frequency of lethals increases much less than

proportionately to rise in dose of ultraviolet, at doses large enough to give the readily detectible frequency of 10 per cent. or more. Thus an increase in dose to nearly 3-fold was found in two series of experiments in this range to cause an increase in frequency to only about 1½-fold, while in another experiment, utilising doses that gave somewhat higher frequencies, an increase in dose to 14-fold caused an increase in frequency to only about 1½-fold. This falling off in mutagenic efficiency with increase in dose appeared both when intensity alone and when time alone were the factors used in changing the dose. However, in experiments in which somewhat lower doses were used than any of those in the experiments above referred to, and in which the radiation was more efficiently and uniformly applied to the polar cap cells, by causing its incidence to be more nearly vertical, the frequency proved to be much more nearly proportional to the dose (a rise in dose to 2·9-fold its former value giving a rise in frequency to 2·4-fold, with the difference between these two ratios less than its own standard error).

There is a series of facts showing that ultraviolet, because of its low penetrating power, is far from evenly distributed among the cells of the polar cap, and that the cells receiving more of it tend to be selectively killed or inactivated. Among these facts are

(1) the far greater mutagenic efficiency of the dose when vertically or nearly vertically applied than when applied from the side ;

(2) the fact that vertical application causes less departure of the mutation frequency-dosage curve from linearity ;

(3) that there is also less departure of the curve from linearity at lower doses than at higher ; and

(4) that a less even application or higher doses are both accompanied by considerable reduction in the effective number of surviving germ cells in the polar cap, as judged by the increase in the relative sizes of clusters of identical mutants.

As the selective damaging of the more illuminated cells—which are the very ones in which more mutations have been induced—would be greater at higher doses, it must result in a part at least of the observed tendency to levelling off of the frequency-dosage curve at higher doses. It could even result in an actual decline of that curve (such as has been noted in some other material) if there were two or more rather discontinuous groups of germ cells, receiving different amounts of illumination. The arrangement of most of the polar cap cells in two layers might provide sufficient discontinuity for this.

If the germ cells differ both in their sensitivity to having their multiplication checked by ultraviolet, and to having mutations induced in them by this agent, and if these two sensitivities are positively correlated, the frequency-dosage curve would be affected

thereby in a manner similar to the effect of the unequal illumination. It does not seem likely however that the polar cap cells differ nearly as much in their sensitivities as in the amount of illumination they receive, when light of 2537 Å is used.

Another circumstance which could result in a continued falling off of the slope of the curve with rise in dose, and its eventual decline, even in case both the degree of illumination and the sensitivities of all the germ cells were identical, would be the existence of a significant amount of photoreparative effect by the ultraviolet in addition to the mutagenic effect of the same wave length. A similar result would be produced if the mutagenic light were accompanied by a sufficient amount of nonmutagenic photoreparative light, the dose of which varied *pari passu* with that of the mutagenic light. It does not seem likely however that there was sufficient difference in the amount of photorepair, in the present experiments, to produce a major share of the observed effects on the mutation frequency-dosage relations.

In the absence of quantitative information concerning the influence of unequal illumination and of photorepair, or of data wherein they are excluded, the present experiments do not decide whether in this material the primary mutagenic action of the ultraviolet is usually produced by one or by more than one activation. However, recent low dosage studies by L. and E. Altenburg and Baker indicate that activations act in combination. Recommendations for further tests are presented.

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A FULLER THEORY OF "JUNCTIONS" IN INBREEDING

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I. THE PLACE OF THE THEORY OF JUNCTIONS

THE theory of junctions was first put forward in the third chapter of my book *The Theory of Inbreeding*, published in 1949. It is set out in Section 14, pages 49-61. Since that time further examination of the problem and new calculations allow the subject to be exhibited in a more complete form, and an inconsequential error to be corrected. I agree with the reviewer (C.A.B.S.) of the first edition, that the subject should have been given a separate chapter. In this paper I hope to give a more complete account of the present state of knowledge.

Each locus of the germ plasm will be represented in the foundation material on a finite number of strands ; for example, on four strands for an inbred line propagated by sib-mating, or by parent offspring mating, but on eight strands for double cousin mating, where two pairs are to be used, or for self-incompatible tetrasomic plants. After n generations more than one strand may be present at some loci, but at an increasing number all but one will have disappeared.

With sib-mating, for example, the probability of not yet being homogenic has been shown to be exactly

$$\frac{1}{10}(5+16\epsilon)\epsilon^n-2^{-n}+\frac{1}{10}(13-16\epsilon)(\frac{1}{2}-\epsilon)^n+\frac{1}{5}.2^{-2n}$$

As, in this expression the second, third and fourth terms are, when n exceeds 10, somewhat trifling in magnitude, and decreasing rather rapidly, it has been convenient to consider the later stages of the inbreeding process in the light of the asymptotic theory, in which the first term only is retained. Out of a total length L, then, the length of strand, on any metric whatever, expected to be still heterogenic will be expressed as

$$l = \zeta\epsilon^n L,$$

where ζ stands for

$$\frac{1}{10}(5+16\epsilon) = 1{\cdot}7944272$$

approximately, and ϵ for

$$\frac{1}{4}(\sqrt{5}+1) = 0{\cdot}80901699.$$

So expressed, the theory supplies the average value of the length of strand still heterogenic, but gives no idea of the extent to which this length may be expected to vary from case to case. This will depend greatly on the number of heterogenic tracts in which the portion still heterogenic is broken up. It is a function of the theory of junctions to supply means of calculating the expected number of such tracts. Indeed, the asymptotic theory may be completed on this basis, since in the limit both the number of tracts and their average length are found to diminish. With tracts widely separated, so that the survival of each is independent of the existence of others, the distribution of tract number must tend to the Poisson limit, for which the frequency of there being n tracts is

$$e^{-m}\frac{m^n}{n\,!},$$

where m is the number expected. Similarly, as the tracts grow shorter by random attrition, the distribution of length among existing tracts must tend to the limit

$$df = \frac{1}{a}e^{-x/a}\,dx.$$

If X stands for the sum of n independent values of x, the distribution of X must be

$$df = \frac{1}{a^n(n-1)\,!}.X^{n-1}\,e^{-X/a}\,dX$$

for any given number n, and for all n possible with the frequencies of the Poisson series the distribution is

$$df = e^{-\frac{x}{a}-m}\sum_{n=1}^{\infty}\frac{1}{n\,!(n-1)\,!}\left(\frac{mX}{a}\right)^n\frac{dX}{X}$$

together with the finite frequency, e^{-m}, at the limit, $X = 0$. The mean value of X in the expression above is easily seen to be ma. Consequently, no more is needed for the complete asymptotic distribution than to supplement the expected length heterogenic with the expected number of heterogenic tracts.

The continuous part of the distribution may be expressed in terms of a Bessel Function, *i.e.* as

$$\frac{m}{\sqrt{Xl}}\,e^{-m\left(1+\frac{X}{l}\right)}\mathrm{I}_1(2m\sqrt{X/l})\,.\,dX,$$

where l is the expected total length heterogenic, and

$$I_1(u) = \frac{1}{i}\,J_1(iu) = \frac{1}{2}u + \frac{1}{2^2.4}u^3 + \frac{1}{2^2.4^2.6}u^5 + \ldots.$$

The expected value of e^{tX} for the distribution

$$\frac{1}{a^n(n-1)\,!}\,X^{n-1}\,e^{-x/a}\,dX$$

is $(1-at)^{-n}$;

and $\sum^{\infty} e^{-m} \frac{m^n}{n!} \cdot (1-at)^{-n}$

is equal to $e^{m/(1-at)-m} = e^{mat/(1-at)}$,

so the Cumulative Function is

$$mat/(1-at) = lt/(1-\frac{lt}{m}),$$

whence, as has been seen, the mean is

$$l = am,$$

the variance

$$\frac{2l^2}{m} = 2ma^2,$$

and the other cumulants $\begin{cases} K_3 = 6ma^3, \\ K_4 = 24ma^4, \\ K_5 = 120ma^5, \end{cases}$

and so on.

In these expressions it should be noted that the condensation at zero is included.

Dr J. H. Bennett has also shown how the variance may be obtained by considering the survival of the heterogenic condition at two loci simultaneously. By this path also he has verified the variance deduced above, both for the case of sib-mating, and for some other cases.

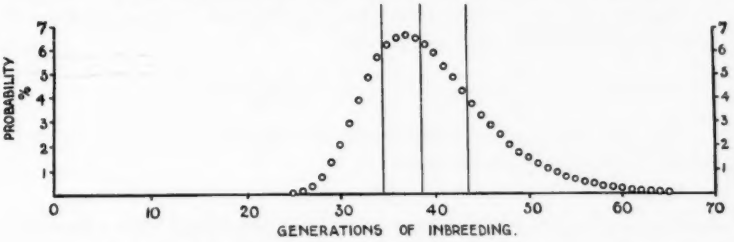

FIG. 1. Probability of elimination of the last remaining Heterogenic Tract at each generation of sib-mating.

2. THE PRODUCTION AND SURVIVAL OF JUNCTIONS

A new junction is formed whenever a crossover occurs in a region for which the parent organism is heterozygous. Since " map length " is a metric defined by the frequency of crossing over, junction-formation will be uniform on this metric. Knowing the frequency of all mating types at each locus, the rate of production of junctions is easily calculated. After production the junction will be inherited like a point-mutation. It may be lost ; in the course of time it may come to prevail in all four strands ; after a given number of generations,

however, it has a calculable chance of being neither lost nor fixed. If it were fixed the strands on both sides of the junction would be homogenic. If they are heterogenic on one side only, the junction must bound a heterogenic tract. If they are heterogenic on both sides, the junction must lie within such a tract. The number of tracts is calculable from the number of external junctions. It is most convenient to calculate first the expected number of all junctions, and then by a more complex argument to calculate the smaller number of internal junctions, the subtraction of which will give the number of external junctions.

The total number of junctions, external and internal, at any stage may be calculated by the method of Section 14. The types of mating capable of giving rise to a new junction at any locus are :

Type	Frequency
$aa = ab$	u
$ab = ab$	v
$ab = ac$	x
$aa = bc$	y
$ab = cd$	z

where a, b, c, d, represent local portions of the kinds of strand available.

Since in types u and y only one parent is heterozygous, only two of the gametes used will come from a heterozygous parent, so that in the four gametes used the rate of occurrence of new junctions per unit map length is

$$2u+4v+4x+2y+4z.$$

This frequency is calculable after any number r of generations of inbreeding, and is found in fact to be

$$\frac{1}{5}(4+24\epsilon)\epsilon^r+\frac{1}{5}(16-24\epsilon)(\tfrac{1}{2}-\epsilon)^r.$$

The first mating involving a junction formed in gametogenesis of the r^{th} generation is of the type

$$je = ee$$

in the $(r+1)^{th}$ generation, where j stands for the strand with a junction, and e for any strand of type a, b, c, d unbroken at this point. The survival of the junction is analogous to that of a point mutation, and the probability of it being neither fixed nor eliminated after $n-r-1$ further generations is

$$\frac{1}{20}(5+16\epsilon)^{n-r-1}+\frac{1}{20}(13-16\epsilon)(\tfrac{1}{2}-\epsilon)^{n-r-1}+\frac{1}{10}\left(\frac{1}{4}\right)^{n-r-1}$$

The expected number of junctions, external and internal at the nth generation is found by multiplying these expressions together and adding for all values of r from 0 to $n-1$. In the summation we use the relations.

$$\sum_{r=0}^{n-1}\alpha^r\beta^{n-r-1}=\frac{1}{\alpha-\beta}(\alpha^n-\beta^n)$$

whenever $\alpha \neq \beta$,

while the sum is $n\alpha^{n-1}$

when $\alpha = \beta$.

The total density of junctions external and internal, evaluated in this way comes to

$$\frac{1}{5}\left\{(8+8\epsilon)n+(428-528\epsilon)\right\}\mathcal{Z}\epsilon^n$$

where the factor $\mathcal{Z}\epsilon^n$ represents the fraction of the total map length heterogenic.

3. INTERNAL JUNCTIONS

The frequency of junctions internal to heterogenic tracts remains to be subtracted.

So far as concerns the leading term, that involving ϵ^n, these are derived in two ways. (i) At a point with chromosomes of only two kinds, of mating type u or v, a crossover may produce a new type of chromosome resembling one kind on the left and the other on the right, which will be an internal junction so long as both the original kinds are still present, but will become external if either of these be lost. Using the analogy of a junction with a mutation, we may think of such mating types as trigenic, of types x or y.

In mating type u (as shown in fig. 2) in which two strands are produced by a heterozygote, one half of the next generation appear

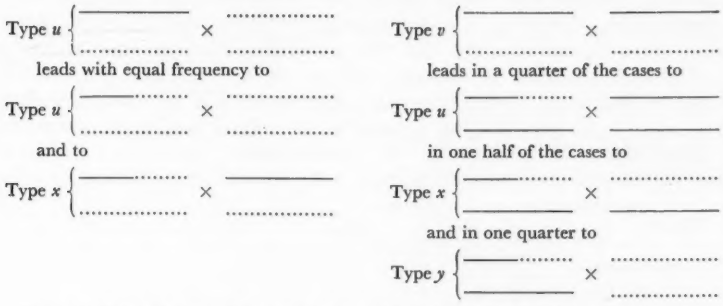

FIG. 2.—Modes of generating trigenic internal junctions from digenic types of mating.

as type x, while from mating type v producing four such strands, one half are of type x and one quarter of type y. The rate of production, per unit map length is therefore

$$u+3v,$$

and substituting

$$u = (20-24\epsilon)\mathcal{Z}\epsilon^r$$
$$v = (40\epsilon-32)\mathcal{Z}\epsilon^r,$$

the rate of production of such trigenic types is seen to be

$$(96\epsilon-76)\mathcal{Z}\epsilon^r.$$

As to their survival, the coefficient both of x_0 and of y_0 in the expectation of $x+y+z$ taken $n-r-1$ generations later is

$$\left(\frac{1}{2}\right)^{n-r-1}.$$

But

$$\sum_{r=0}^{n-1}(96\epsilon-76)\mathcal{Z}\epsilon^r\left(\frac{1}{2}\right)^{n-r-1}$$

$$=(96\epsilon-76)\mathcal{Z}\frac{1}{\epsilon-\frac{1}{2}}\left\{\epsilon^n-\left(\frac{1}{2}\right)^n\right\}$$

So the coefficient of $\mathcal{Z}\epsilon^n$ in the expected number of internal junctions of this type, after n generations is

$$(96\epsilon-76)4\epsilon$$

$$=(96-112\epsilon)$$

$$=5{\cdot}39010$$

(ii) There is also a second type of internal junction with frequency of the same order, which at first I overlooked; for starting with a trigenic or tetragenic mating type, a junction may be formed between two strands, and the junction together with a strand not involved in the junction may both survive as a digenic type in some later generation. Since such a digenic type will thereafter become rarer only as a power of ϵ, the fact that a rapidly decaying type x, y or z is needed to initiate the system, will not prevent internal junctions of this second kind, formed for the most part early in the inbreeding process, from contributing a term in ϵ^n. This process is illustrated in fig. 2·1.

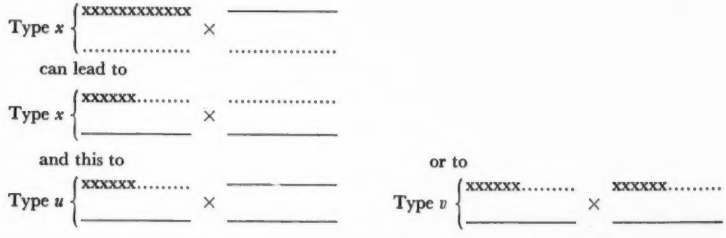

FIG. 2·1.—An illustrative mode of generating digenic internal junctions from trigenic types of mating.

If we let j stand for a junction supposedly between strands a, b, and let c, d stand for strands of different origin from these, the number

of mating types which may be distinguished is 15. Of the three ultimate digenic types, the absolute complexities are already known as follows :—

Mating types		Absolute complexity
$cj = cc, jj$	u	$5+16\epsilon \div 20$
$cj = cj$	v	$6+20\epsilon \div 20$
$cc = jj$	w	$8+24\epsilon \div 20$

A class of mating types which lead only to these apart from other types with the junction lost or external, may thus be evaluated by the frequency with which the types of known complexity are generated. So for four types involving a, c, j the complexities are as follows :—

Mating types	Absolute complexity
$ac = aj$	$2+ 6\epsilon \div 20$
$ac = cj$ or $aj = cj$	$3+10\epsilon \div 20$
$aa = cj$	$1+ 4\epsilon \div 20$
$aj = cc$ or $ac = jj$	$4+12\epsilon \div 20$

The four mating types in c, d, j can also be expressed in terms of the first three.

$cd = cj$	$5+16\epsilon \div 20$
$cc = dj$	$5+16\epsilon \div 20$
$cj = dj$	$6+20\epsilon \div 20$
$cd = jj$	$8+24\epsilon \div 20$

Two types involving a, b, c, j can be expressed in terms of the first seven.

$ab = cj$	$1+4\epsilon \div 20$
$ac = bj$	$2+6\epsilon \div 20$

Finally, the two remaining types involving a, c, d, j can be expressed in terms of the first eleven.

$cd = aj$	$4+12\epsilon \div 20$
$ad = cj$	$3+10\epsilon \div 20$

With these fifteen evaluations it is easy to find the contribution of type $x(ab = ac)$ which out of 4 gametes produced by heterozygotes has

$ac = aj$	$\frac{1}{2}$	worth	$1 + 3\epsilon \div 20$
$bc = cj$	1		$3 +10\epsilon \div 20$
$cj = aa$	$\frac{1}{2}$		$0{\cdot}5+ 2\epsilon \div 20$
$ab = cj$	$\frac{1}{2}$		$0{\cdot}5+ 2\epsilon \div 20$
$ac = bj$	$\frac{1}{2}$		$1 + 3\epsilon \div 20$
with total			$6 +20\epsilon \div 20$

Equally, from type y with two gametes produced by the heterozygote, all will be of type $ac = cj$, giving again,

$$6 +20\epsilon \div 20$$

Finally, from type z of four gametes from heterozygotes, there will be

$ac = cj$	2	$6+20\epsilon \div 20$
$ad = cj$	2	$6+20\epsilon \div 20$

Hence the value from all sources is

$$\begin{aligned}&(6+20\epsilon)(x+y+2z) \div 20\\ &= (6+20\epsilon)L_r \div 20\\ &= (6+20\epsilon)(\tfrac{1}{2})^r L_0 \div 20\\ &= (12+40\epsilon)(\tfrac{1}{2})^r \div 20\end{aligned}$$

since if $z_0 = 1$, $L_0 = 2$.

As before, then, we may evaluate the series

$$\begin{aligned}&\sum_{r=1}^{n-1} (12+40\epsilon)(\tfrac{1}{2})^r \epsilon^{n-r-1} \div 20\\ &= (12+40\epsilon)\frac{1}{\epsilon-\frac{1}{2}}\left\{\epsilon^n-(\tfrac{1}{2})^n\right\} \div 20,\end{aligned}$$

of which the term in ϵ^n is

$$\begin{aligned}&(12+40\epsilon)(4\epsilon) \div 20\\ &= (40+128\epsilon) \div 20\\ &= 4\mathcal{Z}.\end{aligned}$$

To the total of

$$(96-112\epsilon)\mathcal{Z}\epsilon^n$$

internal junctions of the first kind, we have then to add

$$4 \quad \mathcal{Z}\epsilon^n$$

of the second, making

$$(100-112\epsilon)\mathcal{Z}\epsilon^n$$

in all.

Now the total density of junctions was found to be

$$\frac{1}{5}\left\{(8+8\epsilon)n+(428-528\epsilon)\right\},$$

and subtracting the internal junctions, there must be

$$\begin{aligned}&\frac{1}{5}\left\{(8+8\epsilon)n-(72-32\epsilon)\right\}\\ &= \frac{1}{5}(8+8\epsilon)\left\{n-\frac{1}{5}(58-52\epsilon)\right\}\\ &= 2\cdot8944(n-3\cdot1862)\end{aligned}$$

external junctions expected.

The number of separate heterogeneous tracts will, apart from tracts ending on the terminus of a chromosome, be half of this, or

$$1\cdot4472(n-3\cdot1862),$$

and the typical length of such a tract will be

$$\frac{69}{n-3\cdot2} \text{ centimorgans}$$

when n is as great as 10 or so.

Expressions retaining only the leading term are, of course, approximations, and may not be very accurate in the first 10 generations.

For these table 11.0 gives the total number of junctions produced, and the numbers expected at any stage to have been lost, or fixed in tracts already homogeneous, or external, namely at the boundary of a homogeneous with a heterogeneous tract, or finally, internal, within a tract still heterogeneous. These numbers relate to each 100 cM map length.

TABLE 11.0

Fate of junctions produced in the first 10 generations

Gen.	Lost	Fixed	External	Internal	Total produced
1	0·	0·	0·	4·	4·
2	1·	0·	0·75	6·25	8·
3	2·5625	0·0625	1·96875	6·40625	11·
4	4·23438	0·23438	3·09961	5·93164	13·5
5	5·88672	0·51172	3·94434	5·15723	15·5
6	7·43262	0·87012	4·46219	4·36008	17·125
7	8·84253	1·28003	4·69404	3·62090	18·4375
8	10·10516	1·71454	4·70267	2·97763	19·5
9	11·22258	2·15227	4·55113	2·43340	20·35938
10	12·20262	2·57762	4·29324	1·98121	21·05469
	$18-\alpha\epsilon^n(n+\beta)$	$6-\alpha\epsilon^n(n+\gamma)$	$2\alpha\epsilon^n(n-\zeta)$	$\eta\epsilon^n$	$24-\delta\epsilon^n$

$\alpha = (18+58\epsilon)/25 = 2{\cdot}5969194$ $\quad \zeta = (58-52\epsilon)/5 = 3{\cdot}186223$

$\beta = (187-178\epsilon)/5 = 8{\cdot}298995$ $\quad \eta = (26+72\epsilon)/5 = 16{\cdot}849845$

$\gamma = (-13+22\epsilon)/5 = 0{\cdot}959675$ $\quad \delta = (32+112\epsilon)/5 = 24{\cdot}521981$

The rate at which junctions are lost is greatest in the fourth generation, while the rate at which they become fixed reaches its maximum in the ninth. Indeed, for all junctions fixed the average stage of inbreeding is so high as 13 generations. External junctions are highest after 8 generations, and this is the stage at which the number of heterogenic tracts reaches its culmination. Internal junctions are highest after only 3 generations. The table is supplemented by the formulæ in ϵ^n appropriate to later stages.

A distinction may be drawn between end-tracts, terminated by the end of a chromosome, and enclosed tracts. If the total map length is L, and the haploid number of chromosomes is ν we may expect :

End tracts	$2\nu\zeta\epsilon^n$
Enclosed tracts	$\{1{\cdot}45(n-3{\cdot}19)L-\nu\}\zeta\epsilon^n$
Total	$\{1{\cdot}45(n-3{\cdot}19)L+\nu\}\zeta\epsilon^n$.

Since the average length still heterogenic is

$$L\zeta\epsilon^n$$

the standard length of a heterogenic tract may be taken to be

$$1 \div \{1{\cdot}45(n-3{\cdot}19)+\nu/L\}$$

In mice there are 20 pairs of chromosomes, with a total map length roughly estimated to be about 2500 centimorgans. For various

numbers of generations of sib-mating the following table (11.1) shows the expected numbers of tracts of these two kinds, and the expected total map length occupied by them.

TABLE 11.1

Numbers and expected length of heterogeneous tracts

Expected number				Total length cM.	Expected length attached to each dominant cM.
Generations	End tracts	Enclosed tracts	Total number		
5	23·633	47·488	71·121	1477·0	...
10	8·582	49·375	57·957	536·4	18·0
15	2·988	30·432	33·420	186·77	12·25
20	1·035	15·228	16·263	64·71	8·68
25	0·359	6·900	7·259	22·43	6·43
30	0·124	2·954	3·078	7·772	5·29
35	0·043	1·218	1·261	2·693	4·50
40	0·015	0·490	0·505	0·933	3·91

TABLE 11.2

Probability of complete homogeneity at different stages of inbreeding by sib-mating

Generation	Probability	Generation	Probability
25	0·00070	45	0·82005
26	0·00216	46	0·84850
27	0·00565	47	0·87286
28	0·01278	48	0·89360
29	0·02557	49	0·91118
30	0·04603	50	0·92601
31	0·07565	51	0·93847
32	0·11508	52	0·94892
33	0·16386	53	0·95765
34	0·22056	54	0·96492
35	0·28313	55	0·97098
36	0·34910	56	0·97599
37	0·41606	57	0·98019
38	0·48187	58	0·98364
39	0·54479	59	0·98651
40	0·60358	60	0·98888
41	0·65745	61	0·99084
42	0·70601	62	0·99246
43	0·74916	63	0·99379
44	0·78708	64	0·99489
45	0·82005	65	0·99580

From the expected total number of tracts

$$m = \{1\cdot4472(n-3\cdot1862)L+\nu\}\epsilon^n$$

the probability of complete homogeneity is calculated, when m is sufficiently small, as e^{-m}.

With $\nu = 20$, $L = 25$, table 11.2 shows the probability of complete homogeneity from 25 to 65 generations. In fig. 1, p. 189, is shown the distribution of the generation number at which complete homogeneity first occurs. It is probable, however, that the mutation rates at the different loci might supply a new mutation in one of the four strands in every 20 generations or so. Consequently, for long-continued inbreeding we should approach not final homogeneity but a statistical equilibrium in which one or more mutations would occasionally be segregating. The distribution is, of course, a discontinuous one, though bearing some resemblance to the continuous distribution

$$df = d\left(e^{-e^{-x}}\right).$$

Dr Bennett has made a comparative study of these distributions for parent-offspring matings, selfed tetrasomics, and other important types of inbreeding.

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SECONDARY INFLUENCES FROM X-RAY IRRADIATION ON MUTATIONAL PROCESSES IN *DROSOPHILA MELANOGASTER*

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1. INTRODUCTION

In two earlier papers (Bonnier, Lüning and Arnberg, 1952 ; Bonnier and Lüning, 1953) it was shown that recessive sex-linked lethals in the spermatozoan chromosomes of *Drosophila melanogaster* are produced at a higher rate when the sperm is X-rayed in impregnated females than when the irradiation is given to males. In the first of these papers this fact was taken as an indication of a possible indirect effect through the egg cytoplasm of the irradiation. But in the second paper it was found that when the irradiation was given to both males and females, though to the females before mating, this did not result in any increase in percentage of sex-linked lethals in the paternal X-chromosomes.

During the sequence of the experiments Dr Lüning had made much use of the fact that after irradiation of the so-called M_5 (Muller 5) X-chromosome (sc^{S1} *B In-S* w^a sc^8) there occur breaks which manifest themselves as yellow mutations (Belgovski, 1938, 1939). It was, hence, decided to take up this technique for further analyses of the present and allied questions. These studies were made as a joint investigation by Dr Lüning and myself, but since our purposes, and the problems which we wished to elucidate, were somewhat dissimilar we now publish the results separately. The reader is, however, advised to take account also of the paper by Dr Lüning which follows after the present one. The material of our two papers coincides to a large extent. The great majority of the countings and the classifications of the flies were made by Dr Lüning and I am very much indebted to him for the great aid he thus has given me. The irradiation technique is described in his paper. The methods of the crossings for the present investigation are described in the table headings.

2. THE EXPERIMENTS

The experiments were undertaken in three sets. When a first set of experiments was ready the results showed that another set had to be made, and, when this was ready, it was necessary to perform still one more set. In all experiments, females which were homozygous yellow (and besides that, in some experiments, white, singed and in other experiments, achete, scute, prune, singed) were crossed

to *M5* males. The offspring containing aberrations in the paternal X-chromosomes was classified in the following 3 classes. 1. full yellow females; 2. fractional yellow females (which are yellow on a part of the body and grey on the rest; *cf.* discussion); 3. hyperploid males (which contain one maternal and one paternal X-chromosome,

TABLE 1

Progeny from the cross y w sn *females, 2-4 days of age, to* M5 *males. Mode of irradiation was as follows. Series M, only males irradiated; series vF-M, virgin females and males irradiated; series iF-M, irradiation given to impregnated females; series F, only females irradiated. The dose was 3000 r in all series except series F2 in which it was 1000 r. The series M2 and vF-M2 were irradiated together,* i.e. *a group of males was treated under a common X-ray beam and after irradiation the males were separated into two parts of which one was mated to non-treated virgin females (series M2) and one was mated to virgin females which were treated simultaneously with the males (series vF-M2). Two repeats were made within these series. The other series were irradiated separately. The age of the males was 2-4 days at treatment except series M1 in which the males were of 0-1 days of age.*

Mode of irradiation (series)	Full *y* females		Fractional *y* females		Hyperploid males		Total offspring from X-chromosome carrying spermatozoa
	abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4	
M1 . .	20	16	3	2	10	8	12,191
M2 . .	51	20	5	2	26	10	25,695
M3 . .	26	17	7	5	15	10	14,932
Total M .	97	18	17	3	51	10	52,818
vF-M1 .	33	22	13	9	13	9	14,792
vF-M2 .	29	20	10	7	11	7	14,866
Total vF-M	62	21	23	8	24	8	29,658
iF-M . .	10	28	4	11	7	20	3,567
F1 . .	1	...	0	...	0	...	25,967
F2 . .	2	...	0	...	2	...	54,841
Total F .	3	...	0	...	2	...	80,808

Fractionals in series vF-M *vs.* series M, $\chi^2_c = 7{\cdot}2$, P = 0·007.

Fractionals in series vF-M2 *vs.* series M2, exact statistical method, P = 0·02.

the latter, however, consisting of the distal tip and the centromeric region only; they are non-yellow and sterile). Besides these aberrants there occur variegated individuals with a limited number of yellow hairs intercalated between the grey hairs (*cf.* discussion); they are in the tables included among the normal offspring and thus not separately classified. In the progeny of *y ac sc pn sn* females there occurred a small number of yellow variegations, *i.e.* females which

are practically wholly yellow but with a few grey hairs; these are included among the yellow females (see tables 2 and 3).

The irradiations were made in four different ways which will be denoted by the symbols M, vF-M, iF-M and F: 1. irradiation of males only (M); 2. irradiation of males and of virgin females prior to mating (vF-M); 3. irradiation of impregnated females (iF-M); 4. irradiation of virgin females only (F).

In the *first set of experiments y w sn* females, 2-4 days old, were mated to M_5 males the spermatozoa of which were given an X-ray dose of 3000 r at an age of the males of 2-4 days (in one series 0-1 days, see table 1). In conformity with our earlier observations a higher rate of some of the mutational processes is found when the sperm is irradiated in impregnated females than when irradiated in males. The series iF-M was, however, in this case a rather small one and was, accordingly repeated later (table 3). Another very interesting result is seen from a comparison of the rates of fractionals in the two series vF-M and M. As table 1 shows, this rate is in series vF-M more than twice that in series M. A χ_c^2 test (χ^2 with Yates' correction for continuity) gives $\chi_c^2 = 7{\cdot}2$, $P = 0{\cdot}007$, a very good significance, especially as Yates' correction gives somewhat too large a value to P. Now, not all series included in table 1 were made by simultaneous irradiations (see text to the table). If we confine ourselves to the two series M2 and vF-M2 in which the sperm was treated by the very same X-ray beam, the totals are of course diminished and, hence, the level of significance reduced. But applying the exact method, the probability of getting a fit as bad or worse than that found (including both tails) is still as small as 0·02.

The conclusion which thus must be drawn is that irradiation of the virgin *y w sn* females enhances the effect of the irradiation of the M_5 males in the production of fractional yellow daughters. That this is not an effect of the irradiation of the females by itself is concluded from the result of the F series, in which not a single fractional was found in totals of 25,967 (3000 r) and 54,841 (1000 r).

With these conclusions in mind it seemed to be of interest to try to elucidate if the enhancing effect of irradiating virgin females on the production of fractionals from irradiated males was a stable one or if it would disappear after a short while. For this reason a *second set of experiments* was started in which the two modes of irradiation, M and vF-M, were compared in conjunction with a study of the influence of length of time from irradiation of the females to fertilisation of their eggs (table 2). In the series vF-M two different treatments were, therefore, made. In the first, virgin females of 2-4 days of age, were irradiated (3000 r) and immediately thereafter mated to M_5 males, 3-4 days of age, which, under the same X-ray beam, also got 3000 r (series vF-M(2)). For comparison, a lot of the so irradiated males was mated to untreated virgin females, 2-4 days of age (series M(2)). In the second kind of treatment virgin females, 2-4 days

of age were irradiated with 3000 r, thereafter kept without males for a week and then mated to M_5 males, 3-4 days of age which had got 3000 r (series vF-M(9)) ; and, for comparison a lot of these males was mated to untreated virgin females of 9-11 days of age (series M(9)). A series with irradiation of impregnated females (series iF-M) was also included in these experiments. These females were 2-4 days of age and they were irradiated under the same X-ray beam as the corresponding males (see text to table 2).

TABLE 2

Progeny from the cross y ac sc pn sn *females to* M5 *males. Dose in all series 3000 r. A group of males, 2-4 days of age, was irradiated under a common X-ray beam. After irradiation the males were separated into four parts : 1. one part was mated to non-treated virgin females, 3-4 days of age, series M(2) ; 2. one part was mated to non-treated virgin females, 9-11 days of age, series M(9) ; 3. one part was mated to virgin females, 2-4 days of age, which had been subjected to the same X-ray beam as the males, series vF-M(2) ; 4. one part was mated to virgin females, 9-11 days of age, which were given 3000 r a week earlier, series vF-M(9). The males were taken away from the females one day after beginning of mating. The very same males had, during the day before their irradiation been mated to virgin females, 2-4 days of age, and these females were then irradiated together with the males under the same X-ray beam, series iF-M. The series M(2), vF-M(2) and iF-M were repeated for a total of 4 times, and the series M(9) and vF-M(9) for a total of 5 times.*

Mode of irradiation (series)	Full *y* females		Fractional *y* females		Hyperploid males		Total offspring from X-chromosome carrying spermatozoa
	abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4	
M(2) . .	21*	29	3	4	20	27	7,365
M(9) . .	35†	29	3	3	21	18	11,878
Total M .	56	29	6	3	41	21	19,243
vF-M(2) .	31†	28	4	4	15	14	10,971
vF-M(9) .	37*	20	10	5	45	25	18,294
Total vF-M	68	23	14	5	60	21	29,265
iF-M . .	60	27	8	4	63	29	22,102

* Includes 2 females which had a few grey hairs.
† Includes 3 females which had a few grey hairs.

As mentioned above the females of the first set of experiments (table 1) were *y w sn*. The reason for the choice of this strain was that we had used it in several earlier experiments and, hence, knew that it was well suited for our purposes. From the point of view which governed Dr Lüning's purposes it was, with the results of the first set of experiments in mind, of interest to use more markers in the left tip of the X-chromosome (see his paper). And as, from

the points of view of the present studies, the only necessity seemed to be that the females were yellow, it was agreed that, in the second set of experiments, the females should be taken from a *y ac sc pn sn* stock. In these experiments it was thus such females which were crossed to M_5 males.

From table 2 it is found that neither the age difference between the females nor the difference in length of time between irradiation of the females and laying of their eggs produced any appreciable differences in the rates of aberrants. The most surprising result, which at first seemed to contradict our earlier observations, is that when the rates of fractionals are compared for the three modes of

TABLE 3

Comparison of the progeny from y w sn *and from* y ac sc pn sn *females after crossing to* M_5 *males. One part of a group of 3-4 days old males was mated to virgin* y w sn *females and one part to virgin* y ac sc pn sn *females, all females of 2-4 days of age. One day later these females and males, together with virgin females of the same two strains and of the same age were irradiated with 3000 r under a common X-ray beam. The offspring of the females which were impregnated at irradiation form the series iF-M. The offspring of the other females form the series vF-M. All series were repeated for a total of 5 times.*

Females to which the males were mated	Mode of irradiation (series)	Full *y* females		Fractional *y* females		Hyperploid males		Total offspring from X-chromosome carrying spermatozoa
		abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4	
y w sn .	vF-M	35	26	1	1	14	10	13,403
y ac sc pn sn	vF-M	19	25	1	1	16	21	7,473
y w sn .	iF-M	43	27	15	10	31	20	15,702
y ac sc pn sn	iF-M	18*	39	1	2	14	30	4,621

Fractionals from series *y w sn* vF-M this table *vs.* corresponding series table 1, $\chi_c^2 = 6{\cdot}9$, $P = 0{\cdot}009$.

* Includes 2 females which had a few grey hairs.

irradiation, the rates from irradiation of the males only (M) are neither increased by irradiation of the virgin females (vF-M) nor by irradiation of the sperm in impregnated females (iF-M). The question therefore arose if the " nature " of the females which are crossed to the M_5 males is of influence in the production of aberrants from irradiated male gametes. A new set of experiments was, accordingly, started.

In this *third set of experiments* (table 3) only the two modes of irradiation were made : irradiation of M_5 males, 3-4 days of age, together with irradiation of virgin females, 2-4 days of age (vF-M) ; and irradiation of impregnated females (iF-M). The two strains of females *y w sn* and *y ac sc pn sn* were used (see text to table 3). Here it is found that, as regards the production of fractionals in the iF-M

series, the high rate is again restored in the progeny of *y w sn* females (10 per 10^4) and the low rate in the progeny of *y ac sc pn sn* females (2 per 10^4). Because of the relative smallness of the series iF-M from the last type of females, the difference between the two iF-M series is not significant, but is, to say the least, strongly indicative. Another point of surprise is the low rate of fractionals in the vF-M series after *y w sn* females (1 in 13,403) as compared with the corresponding rate in table 1 (23 in 29,658). This difference is significant, $P<0{\cdot}01$. Now, as all rates *within* similar types of series, except the last mentioned one, are of the same order of magnitude, it is quite justified to pool the figures. This is done in table 4.

TABLE 4

Totals from tables 1, 2, 3

Females to which the males were mated	Mode of irradiation (series)	Full *y* females		Fractional *y* females		Hyperploid males		Total offspring from X-chromosome carrying spermatozoa
		abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4	
y w sn .	M	97	18	15	3	51	10	52,818
y ac sc pn sn	M	56	29	6	3	41	21	19,243
y w sn .	vF-M	97	23	24	6	38	9	43,061
y ac sc pn sn	vF-M	87	24	15	4	76	21	36,738
y w sn .	iF-M	53	28	19	10	38	20	19,269
y ac sc pn sn	iF-M	78	29	9	3	77	29	26,723
y w sn .	F	3	...	0	...	2	...	80,808

Comparison between iF-M and (vF-M+M)

y w sn series *y* females, $\chi^2_c = 3{\cdot}6$, $P = 0{\cdot}06$
fractional *y* females, $\chi^2_c = 9{\cdot}8$, $P = 0{\cdot}002$
hyperploid males $\chi^2_c = 14{\cdot}9$, $P = 0{\cdot}0001$

y ac sc pn sn series *y* females, $\chi^2_c = 0{\cdot}77$, $P = 0{\cdot}38$
fractional *y* females $\chi^2_c = 0{\cdot}006$, $P = 0{\cdot}94$
hyperploid males, $\chi^2_c = 4{\cdot}5$, $P = 0{\cdot}03$

Comparison within iF-M, between *y w sn* and *y ac sc pn sn* series

y females, $\chi^2_c = 0{\cdot}06$, $P = 0{\cdot}80$
fractional *y* females, $\chi^2_c = 6{\cdot}7$, $P = 0{\cdot}009$
hyperploid males, $\chi^2_c = 3{\cdot}4$, $P = 0{\cdot}06$

From this table it is seen that in the offspring of *y w sn* females the higher rates of both fractionals and hyperploids from impregnated females (iF-M) as compared with the rates from the other two modes of irradiation (excluding series F) is clearly significant. In the case of the offspring of *y ac sc pn sn* females there is no difference in the rates of fractionals in these three modes of irradiation. And, when a comparison is made of the rates of fractionals from impregnated females between the *y w sn* and the *y ac sc pn sn* series, this difference is also significant. From this it must be concluded that the " nature " of the strain from which the mothers of the fractionals come is of

influence upon the rates of these fractionals. From table 4 it is also seen that the rate of hyperploids is higher in the offspring of *y ac sc pn sn* females than in the offspring of *y w sn* females. But as this is so regardless the mode of irradiation, this has nothing to do with the questions dealt with in the present article. The causes here at work are discussed by Lüning (1954).

3. DISCUSSION

The phenotype of the fractional yellow females is, as pointed out earlier, partly yellow and partly grey. They should, however, be clearly distinguished from variegated yellow of the mottled type. These variegates are of two kinds. The majority have a limited number of yellow hairs intermingled between the grey hairs, their general appearance is grey and they may be found in the progeny of crosses between *y* females and M_5 males also without irradiation. These variegates are not separately classified in the tables but included in the totals. The second type of variegates is practically wholly yellow with a few grey hairs. They are in the tables included among the yellow females but their numbers are given in footnotes.

The fractional yellow females are not mottled. There is on them a sharp borderline between yellow and grey. The body surface is usually divided into two parts, one yellow and one grey, but may also be broken up into more parts which are alternatingly yellow and grey. Though the fractionals are wholly female the difference between variegates and fractionals may be compared to the difference between intersexes and gynandromorphs. The mosaicism of a variegated or of an intersex is not due to dissimilarities in the genotypic make-up of their cells. The mosaicism of a fractional or of a gynandromorph is, on the contrary, due to a difference in the chromosomal constitution in the different parts of the body : in gynandromorphs one part of the body is female and one part male ; in fractionals one part is heterozygous for yellow and one part homozygous.

The truth of this distinction between fractionals and variegates should be ascertainable by testing from their progeny : The M_5-carrying daughters of a variegated should be variegated, whereas such daughters of a fractional should be wholly grey or, sometimes, wholly yellow. Only three of the female aberrations were actually progeny tested by crossing them to brothers. The first of these was a yellow variegated (yellow Bar with a few grey hairs) from series vF-M(9), table 2. Here progeny consisted of 17 *y B* and 34 *y ac sc pn sn* daughters, 24 *y ac sc pn sn* and no *B* sons. The *y B* daughters were variegated in that they had a few grey hairs. The second was a fractional yellow female from series M(2), table 2. Of her daughters 40 were *B*, 28 *y ac sc pn sn*, 1 *y ac B* ; and of her sons 24 M_5 and 23 *y ac sc pn sn*. The third was a fractional from series iF-M, table 2 : it was only noted that her offspring showed her ovaries to be

M5/y ac sc pn sn. So far as these three progeny tests go they conform thus to the distinction made between fractionals and variegates.

For the discussion it is of primary importance to remember that the types of aberrations included in the tables are all due to mutational processes within the *paternal* X-chromosomes. It follows that if the rates of some or more of these aberrations are influenced by the nature of the strain from which the mothers are taken and/or by differences in treatment of the mothers, such influences must be brought about by secondary pathways. Furthermore, we know that the majority of the yellow daughters from the cross of yellow females to *M5* males with irradiated spermatozoa is due to breaks in the *y*-region of the *M5* chromosome (see Lüning, 1952). We must, therefore, conclude that a fractional yellow daughter from the same cross is due to a break in that region, but in only one of the chromosome's two chromatids.

The results of the statistical analyses of the preceding section may be summarised as follows.

1. If among the progeny of *y w sn* females a comparison is made between the number of aberrations, on the one hand, when irradiation of the spermatozoa is made on impregnated females (iF-M) and, on the other hand, when the spermatozoa are irradiated in the males (vF-M and M), a clearly significant heterogeneity is found. This difference in the rates is very marked for fractionals and hyperploids but it is perhaps questionable whether there is any true difference in the rates of yellow females.

2. In the progeny of *y ac sc pn sn* females the corresponding difference in rates between iF-M and (vF-M and M) is restricted to the hyperploids.

3. If a comparison is made of the rates of fractionals *within* the iF-M series, *between* the progenies of *y w sn* females and of *y ac sc pn sn* females, the difference is significant.

4. When the rates of fractionals within the series in which the spermatozoa were irradiated in the males are compared, on the one hand when combined with irradiation of the virgin females (vF-M) with, on the other hand when the males only are irradiated (M), a significant difference was, at one occasion, found in the progeny of *y w sn* females.

5. This difference was not found when a repeat of the experiment was made at a later occasion ; and it was not found in the progeny of *y ac sc pn sn* females.

6. Irradiation of virgin females only does not increase the rates of any of the aberrations above the spontaneous rates.

The statement under point 1 agrees exactly with our findings concerning the production of sex-linked lethals (Bonnier and Lüning, 1953). We found there that when crossing *y w sn* females to *M5* males and irradiating the sperm, a higher rate of sex-linked lethals was produced if the sperm was irradiated in impregnated females

than if it was irradiated in the males. A small-scale experiment showed that the same result occurred if wild type Canton S males were used instead of M_5 males. As a possible explanation we suggested that the spermatozoa are in a more sensitive state when stored in the somewhat more movable condition in the females than in the more rigid bundles within the testes. The statement under point 3 shows, however, that the differences cannot be solely due to changes in states of the spermatozoan nuclei. It must be supposed that the " nature " of the females, in the progeny of which the aberrations occur, also exerts an influence.

We will discuss three possible directions in which the explanation may be thought to be sought. In the first place the differences may be due to different oxygen tensions. Several investigators have shown that if X-ray irradiation is made in gas of lower oxygen concentration than in air, the number of chromosome breaks will be reduced (see Hollaender, Baker and Anderson, 1951 for review). It seems, however, not to be finally settled if this effect is due to a decrease in the number of primary breaks or to an increase in the number of restitutions of broken ends (Baker and Edington, 1952 ; Baker and von Halle, 1953). If now the differences found in the rates of the present study are due to differences in oxygen tension it would be necessary to suppose that, though all our experiments are made in air, the receptacles of *y w sn* and of *y ac sc pn sn* females have different oxygen tensions. It would also be necessary to suppose that these differences act selectively on different types of aberrations.

As a second way of explanation we may think of chemical protection. It is known that a number of chemical compounds protect against irradiation damage (see Hollaender, Baker and Anderson, 1951 for review). It would then be thinkable that there are different amounts of protecting stuffs in the walls of the testes and of the receptacles, and that such differences also are to be found between the receptacles of females from different strains. It would, however, still be difficult to explain why irradiation of the spermatozoa within the females increases the rate of fractionals and of hyperploids in the progeny of *y w sn* females but only of hyperploids in the progeny of *y ac sc pn sn* females.

The third way of explanation takes differences in energy levels into account. During the last few years different investigators have, especially in connection with studies on the increasing effect on chromosome breaks from infra-red irradiation when combined with X-ray irradiation in *Drosophila* and *Tradescantia*, described the phenomenon in terms of such levels and of surmountings of energy barriers (Swanson and Hollaender, 1946 ; Swanson, 1947, 1949 ; Yost, 1951 ; Swanson and Yost, 1951 ; McElroy and Swanson, 1951 ; Yost, 1952). The energy coming (indirectly) from an X-ray quantum may, according to this view, sometimes be too weak to produce a break but the chromosome molecule in question may still

have absorbed an amount of energy not much below what is necessary for surmounting the barrier, *i.e.* for producing a break. This state of the molecule may be metastable, *i.e.* the molecule may remain for a considerable time in an energy valley situated at a higher level than the initial state. If this valley is not far below the peak of the energy barrier and if an extra amount of energy, which may be too small to have any effect by itself, is added, for instance by infra-red irradiation, the molecule may jump over the barrier and the break will be fulfilled. Now, applying such considerations to our cases, we should suppose that X-ray irradiation of the fluid in the receptacles (or perhaps of some other female tissue) gives to its molecules a supply of energy which, by secondary paths, may be added to the energy of the irradiated spermatozoan chromosome loci. The X-ray irradiation of the females and, according to this hypothesis, the supposed production thereby of an energy source should thus here correspond to the infra-red irradiation.

One of the results of the present study could perhaps be explained on the assumption of the validity of the energy level hypothesis. In point 4 it was stressed that the rate of fractionals was, in the first set of experiments, enhanced by combining irradiation of the males with irradiation of the virgin females. The fractionals are produced when the paternal X-chromosomes have one chromatid broken and one unbroken. Such a thing may happen in different ways. But for those cases which constitute the surplus when the rate in the vF-M series is compared to that in the M series of table 1, we may imagine that, from a primary process, a suitable locus of the chromosome is raised to a metastable state not far below the peak of the energy barrier. If this state could remain to after splitting there would be two homologous loci of which one or both could be excited. Now, it would seem to be much more likely that an extra amount of energy (from a secondary source produced by irradiating the female tissues and enough for the production of a break) would be absorbed in one only than in both of these loci. And this is the same as to say that one is much more likely to find an increase of fractional yellow females than of full yellow females whether both or only one of the chromatid loci remained in the metastable state.

Whichever of the explanations we may choose there will remain several unexplained experimental facts. It seems to me that the energy level assumption gives the best fit to the differences in the rates of fractionals between the vF-M and the M series from table 1. But it is very probable that other circumstances are influential in causing the differences found with regard to the other types of aberrations, and that possibly also different forces are at work which interact in the production of the end result.

4. SUMMARY

1. In a preceding article it was shown that the rate of X-ray induced sex-linked lethals in sperm of *Drosophila melanogaster* is increased if the sperm are irradiated in impregnated females when a comparison is made with the result of irradiation of the males. The present study shows that the same is true with regard to other kinds of X-ray induced aberrations in the male gametes containing the M_5 X-chromosome, viz. aberrations which after crossing to yellow females produce hyperploid sons, fractional yellow daughters and possibly also full yellow daughters.

2. It is shown that this effect is influenced by the nature of the strain of yellow females to which the M_5 males are crossed, insofar as the frequencies of the fractional yellow daughters are significantly different when comparison is made between two such strains.

3. In one series of experiments it was found that the rate of fractional yellow daughters produced by irradiation of males was significantly increased if the females were irradiated prior to mating. In a repeat of the same type of experiment, performed at a later occasion, no such increase was found; but comparing the rates of fractional yellow daughters which were found on the two occasions, the difference was found to be statistically significant. Any influence of irradiating the females only was not found.

4. Three possible ways of causing these results are discussed, *viz.* differences in oxygen concentration, differences in chemical protection, and a system of metastable energy levels.

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VARIATIONS IN THE BREAKABILITY OF CHROMOSOMES IN MATURE SPERMATOZOA OF *DROSOPHILA MELANOGASTER* AT DIFFERENT MODES OF IRRADIATION

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1. INTRODUCTION

In a preceding study (Bonnier and Lüning, 1953) it was found that a higher rate of recessive lethals is induced when the irradiation is given to spermatozoa stored in impregnated females than when the irradiation is given to males. It was further demonstrated that this is not due to the irradiation *per se* of the females but may perhaps be due to a difference in the state of the spermatozoa when within the seminal receptacles of the females as compared with its state within the males. This supposition is based on analogy with differences in the state in various stages of spermiogenesis already described (Lüning, 1952*a* and *b*). In these papers it is demonstrated that the breakability of the chromosomes depends on the age of the males and the stages in spermiogenesis at treatment. It was further shown that apparent gene mutations, known to be *inter*genic changes, vary as the rate of breaks while other gene mutations, visibles and recessive lethals, may vary but not as much as the rate of breaks. From these data it was concluded that there exist both *intra-* and *inter*genic changes and that recessive lethals are of both types. The rate of *intra*genic changes did not seem to be changed in spermiogenesis. Hence the difference in state may be confined to the breakability of the chromosomes with a consequent increase in the rate of *inter*genic changes.

The higher rate of recessive lethals in spermatozoa irradiated in impregnated females versus in males may be due to an increase in *intra-* and/or *inter*genic changes. As the existence of *intra*genic changes can be proved only in an indirect way one must first test the occurrence of *inter*genic changes in spermatozoa irradiated in impregnated females versus in males. The present study deals with this analysis.

2. TECHNIQUE

When investigating the problem of the different states of the spermatozoa in spermiogenesis two types of aberrations both due to *inter*genic changes were used, *viz.* hyperploid males, deficient in nearly the whole paternal X-chromosome except the extreme tip and probably the centromere region (Lüning, 1952*a*), and *yellow* mutations in the sc^{S1} *B InS* w^a sc^8 (Muller-5, *M5*) chromosome which according to Belgovsky (1938, 1939) are due to minute rearrangements (Lüning, 1952*b*). Among the yellow mutations there appeared cases in which parts only were yellow —so-called fractionals. These aberrations were of special interest to Dr Bonnier

and as our problems were somewhat dissimilar we publish the results separately. In this paper the fractionals are included in the tables but will not be further discussed.

By using these two types of aberrations induced in spermatozoa irradiated in impregnated females or in males it is possible to examine the rates of *inter*genic changes in the two cases.

Following this principle *y w sn* females have been mated to M_5 males either before or after irradiation. Among the offspring from these crosses there appeared *yellow* females (full and fractionals) and hyperploid males which were easily recognisable. To ensure that the results obtained were not due to some special features of the M_5 stock the experiment was repeated with *y w sn* females mated to *Canton S* males. The result of the latter experiment led to analysis of the offspring from *Canton S* males mated to *y ac sc pn sn* females. This in its turn led to another experiment with *y ac sc pn sn* females mated to M_5 males.

In a fifth group of experiments the rate of induced losses of X- and Y-chromosomes was studied when the spermatozoa were irradiated in the impregnated females and in the males. A special technique was developed in this experiment making use of a Y-chromosome containing a small part of an X-chromosome, viz. $sc^8\,Y$, kindly put to my disposal by Dr Muller. This chromosome contains the wild type allele of the *yellow* locus giving males with *y w sn* alleles in the X-chromosome the phenotype " white singed " with wild type body colour. In these experiments *y w sn* females (known to be free from extra Y-chromosomes) were mated to $M_5 : sc^8\,Y$ males. Among the offspring there appeared XO males classifiable as *y w sn* among the normal XX and XY offspring with wild type body colour. This technique will be further discussed below.

It was previously shown (Lüning, 1952*a*) that the rate of chromosome breaks induced in mature spermatozoa was about the same when 2-3 or 6-7 days old males were irradiated, but lower if the males were 0-1 or 1-2 days old. For that reason the males were chosen which were 2-3 days old when first mated or 2-4 days old when irradiated. This is best elucidated by an example from an experiment consisting of three modes of irradiation, males only (series M), virgin females and males (series vF-M) and impregnated females (series iF-M) : 2-3 days old males were mated to females during 24 hours. Then they were etherised and irradiated together thus ensuring that spermatozoa in males and in impregnated females got the same dose. Under the same beam there was a separate group of virgin females. Immediately after irradiation the females and males were separated. The impregnated females were placed in vials without males (series iF-M). The males were divided into two lots, one lot was mated to the irradiated virgin females (series vF-M) and the other lot to non-irradiated virgin females (series M). In series M and vF-M the males were never allowed to stay with the females more than a maximum of 6 days. In some experiments they were discarded already one day after mating began. By this mating and irradiation technique one could be sure that equivalent spermatozoa were used in the different series.

In the first part of the investigation the irradiations were made at the Radiophysical Institute by means of a Siemens Stabilivolt apparatus of 155 kV, 6 mA and 1 mm. Al. In the second part, our new Skandia intensive apparatus was used, operated at 170 kV, 15 mA with an inherent filtration equivalent to 2 mm. Al. The dose was 3000 r given in 6 min. in both apparatuses. Stocks and cultures were reared on yeast suspension as described by the author (1952*a*). They were kept in the incubator at 25° ± 1° C.

3. THE EXPERIMENTS

1. *y w sn* ♀♀ × M_5 ♂♂

As the purpose of the present study was to investigate the mechanism underlying the higher rate of recessive lethals in spermatozoa

irradiated in impregnated females versus in males, the same stocks were used in the first analysis of the *inter*genic changes (*yellow* mutations and hyperploid males) as in the previous study on recessive lethals. The three modes of irradiation used were spoken of above and also at the head of table 1, where the results are presented. Each series is a summation of two or three experiments carried out at different periods in late 1952 and early 1953. There was no difference in the rate of full yellow mutations and hyperploid males between the experiments in each series. For that reason only the pooled data of each series is given in table 1.

TABLE 1

Offspring from crosses of y w sn ♀♀ × M5 ♂♂, *mated at an age of 2-4 days. In the first series males only were irradiated, series M. In the second series both the males and the virgin females were irradiated, series vF-M. In the third series the females were irradiated after impregnation, series iF-M. Series M consists of 2 experiments carried out at different times, series vF-M of 3 experiments and series iF-M of 2 experiments. One experiment in series M was carried out parallel to one in series vF-M. Another in series vF-M was carried out parallel to one in series iF-M. There do not exist any differences, as checked by* χ^2, *among the different experiments in each series as concerns full* yellow *mutations and hyperploid males. For that reason there are given the totals only of each series. The dose was 3000 r.*

Series	Total offspring from X-carrying spermatozoa	Yellow		Fractional yellow		Hyperploid males	
		abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4
M . .	40,627	77	19	12	3	41	10
vF-M .	43,061	97	23	24	6	38	9
iF-M . .	19,269	53	28	19	10	38	20

A heterogenity test on the pooled rates of full *yellow* mutations and hyperploid males in the three series gives $P = 0{\cdot}001$. A comparison between series M *vs.* series vF-M gives $0{\cdot}70 > P > 0{\cdot}50$, and series M and series vF-M *vs.* series iF-M gives $P < 0{\cdot}001$. A comparison of the *yellow* mutations in series M and vF-M *vs.* series iF-M gives $0{\cdot}10 > P > 0{\cdot}05$. A similar comparison of hyperploid males gives $P < 0{\cdot}001$. As the rates of both *yellow* mutations and hyperploid males vary in the same direction we may conclude that there is induced a higher rate of *inter*genic changes in spermatozoa irradiated in females than in males.

2. *y w sn* ♀♀ × *Canton S* ♂♂

In order to study if the increased rate of *inter*genic changes obtained in spermatozoa irradiated within impregnated females versus in males was a peculiarity in the *M5* stock the experiments were repeated

with the Canton S stock. The result of the three series is presented in table 2. The use of the Canton S stock may also give some indications of a possible effect on *intra*genic mutations as *yellow* mutations in the wild type stock are supposed to be mostly due to *intra*genic changes (Lüning, 1952*b*). As is seen in table 2 the rate of *yellow* mutations is in fact lowest in series iF-M. There is thus no indication of an increased rate of *intra*genic changes. The rate of *white* mutations which (according to the author, 1952*b*) are due to both *intra*- and *inter*genic changes is, however, significantly ($P = 0{\cdot}001$ by χ^2_c test) higher in series iF-M than in series M. Also the *singed* mutations

TABLE 2

Offspring from crosses y w sn ♀♀ × Canton S ♂♂. *Both females and males were mated at an age of 2-4 days. The same series were carried out as in table 1. In series M is included two experiments carried out at different times. One of them was parallel to series vF-M and series iF-M. There were no differences between the two experiments in series M, as checked by* χ^2, *for that reason they were pooled in the table. The new fractional mutations which appeared with about the same frequency in the three series are figured as full mutations in the table. White variegated mutations are not listed among white mutations.*

Series	Total offspring from X-carrying spermatozoa	Yellow		White		Singed		Gynandromorphs		Hyperploid males	
		abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4
M	78,347	17	2	23	3	12	2	50	6	50	6
vF-M	40,251	8	2	15	4	7	2	34	8	26	6
iF-M	27,186	4	1	22	8	8	3	22	8	35	13

showed an increase, although it is not significant. The rates of gynandromorphs seem to be uninfluenced by the different modes of irradiation. This agrees with the result obtained by the author (1952*a*) when treating various stages in spermiogenesis. The rates of hyperploid males show the same variation as that observed in the series with M_5 males. Hence we may conclude that the increase rate of *inter*genic changes in spermatozoa treated within impregnated females versus in males was not a peculiarity to the M_5 chromosome but appeared also in the wild type chromosome used, Canton S. In an addendum to Bonnier and Lüning (1953) it was shown that the rate of recessive lethals was higher in iF-M type than in M-type of irradiation of Canton S spermatozoa.

3. *y ac sc pn sn* ♀♀ × *Canton S* ♂♂

The strong increase in the rate of white mutations in series iF-M in table 2 seemed strange in comparison with the moderate increase in the rate of hyperploid males, the more so because the rate of white

mutations had been shown to vary with the stages in spermiogenesis but not as much as the rate of breaks (Lüning, 1952*b*). One possible explanation of the high rate of white mutations is a random variation due to the small absolute number. Another explanation is a localised breakability in the *white* region. In order to test this the experiment described in section 2 was repeated using females with other marker genes, viz. *y ac sc pn sn*. Any localised increase in the rate of *inter*genic changes in the *white* region would be detected by an increase in the rate of hyperploid males phenotypically *sn* compared to *pn sn* hyperploid males in which the left break was to the left of *pn* at locus 0·8.

TABLE 3

Offspring from crosses y ac sc pn sn ♀♀ × Canton S ♂♂. *Both females and males were mated at an age of 2-4 days. The two series M and iF-M were carried out parallelly. The hyperploid males were of two phenotypic classes, viz.* pn sn *and* sn.

Series	Total offspring from X-carrying spermatozoa	Yellow		Singed		Gynandromorphs		Hyperploid males				Total per 10^4
								pn sn		*sn*		
		abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4	
M	29,862	4	1	6	2	12	4	26	9	4	1	10
iF-M	32,278	7	2	10	3	23	7	59	18	9	3	21

This experiment consisted of two series only, viz. M and iF-M. The result is presented in table 3. There is no indication of an increase in the *inter*genic changes in the *white* region compared to the region to the left of *pn*. There was, however, a difference in the total rate of hyperploid males in table 3 as compared to that in table 2. Males of the same stock have been irradiated and mated to females of different stocks. The cause of the higher rate of hyperploid males in the experiment in table 3 is not due to a higher viability of individuals having a greater part of the distal end of the paternal X chromosome than in the experiment in table 2. This result suggested a fourth series of experiments, viz. *y ac sc pn sn* females mated to M_5 males.

4. *y ac sc pn sn* ♀♀ × M_5 ♂♂

Besides studying the rates of hyperploid males induced in M_5 spermatozoa fertilising *y ac sc pn sn* eggs after different modes of irradiation it was of interest to Dr Bonnier to study the dependence of fractional *yellow* mutations of the time from irradiation to fertilisation. For that reason in the series where virgin females and males were treated the females were mated either immediately after irradiation or were stored 7 days without males before mating. As

a check the males in the series with irradiation of males only were mated to 2-4 and 9-11 days old females. In the series in which impregnated females were irradiated 2-4 days old females only were used. As regards the full *yellow* mutations and hyperploid males no difference was found between the two ages of females. For that reason they are combined in table 4.

A comparison of the *yellow* mutations shows no significant difference, $0{\cdot}50>P>0{\cdot}30$ between the three series. The rate of hyperploid males is, however, significantly higher in series iF-M than in series M and vF-M, $0{\cdot}05>P>0{\cdot}02$. Comparing the rates of hyperploid

TABLE 4

Offspring from crosses y ac sc pn sn ♀♀×M5 ♂♂. *In series M the males were irradiated at an age of 2-4 days and mated to 2-4 or 9-11 days old females. In series vF-M the males were irradiated at an age of 2-4 days and mated to females (irradiated at an age of 2-4 days) either immediately or one week after treatment of the latter. In series iF-M the females were mated at an age of 2-4 days and irradiated when impregnated. The main part of the three series was carried out parallelly. Later was added data to series vF-M and iF-M. There was no heterogenity between the experiments within the different series, as checked by* χ^2.

Series	Total offspring from X-carrying spermatozoa	Yellow		Fractional yellow		Hyperploid males	
		abs. nr.	per 10^4	abs. nr.	per 10^4	abs. nr.	per 10^4
M . .	19,243	56	29	6	3	41	21
vF-M .	36,738	87	24	15	4	76	21
iF-M . .	26,723	78	29	9	3	77	29

males in tables 1 and 4 one sees that the rate is higher in all series in table 4. It is consequently of no importance that the females were irradiated in some series and not in others. A possible cause of this difference will be discussed below.

5. *y w sn* ♀♀×*M5* ; *sc*^{8} *Y* ♂♂

In the preceding sections it has been demonstrated that a higher rate of rearrangements is induced in spermatozoa irradiated in impregnated females than in males. This difference could be due either to a different rejoinability of broken ends or to induction of different rates of breaks in the three modes of irradiation. It was consequently of interest to discriminate between these two possible causes. Such an analysis must be based on the rate of breaks which remain unjoined since the preceding experiments were based on rejoined broken ends.

Muller (1940) and Pontecorvo (1942) showed that the rate of

losses of X- and Y-chromosomes increased linearly with the dose and they suggested that this was due to single breaks whose ends did not combine but remained unjoined until splitting when there was a sister union giving one dicentric chromosome and one acentric fragment which were likely to be lost in the nest cell division. Hence a study of losses of X- and Y-chromosomes would give a measure of the rate of breaks which remain unjoined in the different modes of irradiation.

Before discussing the technique and results of the experiments we ought to consider *a priori* what types of result we may obtain according to the two hypotheses proposed above.

1. Supposing that the same rate of breaks is induced by the three modes of irradiation but that in one series (iF-M) the rate of rearrangements is greater than in the other two (M and vF-M) one must expect that the rate of breaks which remain unjoined and/or restitute in the original order would decrease.

1A. If it is further supposed that it is merely a matter of chance if broken ends will recombine or remain unjoined one would, in this case, expect a lower rate of X- and Y-chromosome losses in series iF-M than in series M.

1B. If it is supposed that different kinds of breaks are induced as Kaufmann and Wilson (1949) suggested, viz. complete and potential where the former eventuate in sister chromatid union with a subsequent elimination of the chromosome broken; and the ends of potential breaks joined in the original or in a new order, one would not expect any considerable difference in the rate of X- and Y-chromosome losses in series iF-M and M. The existence of the two classes of breaks, complete and potential, has, however, not been verifiable by me (1952*c*) when comparing the different types of aberrations studied in various stages of spermiogenesis.

2. Supposing that the rate of induced breaks is higher in series iF-M than in series M and vF-M one would expect an increase in the rate of unjoined breaks which will be detected by an increase in the rate of losses in X- and Y-chromosomes in series iF-M versus series M.

In order to discriminate between the two hypotheses discussed under 1 and 2 the following technique was worked out:

y w sn females which were guaranteed to be free from extra Y-chromosomes were used. This was ensured by keeping the *y w sn* stock with males having the sc^8 *Y* chromosome. These males are phenotypically *w sn*. They were mated to *y w sn* females for several generations. If there appear XXY females they are phenotypically *w sn* and thus when using females phenotypically *y w sn* they must be free from extra Y-chromosomes. These females were mated to M_5; sc^8 *Y* males. The normal F_1 offspring is non-yellow. Losses of the paternal X or of the Y-chromosome manifest themselves as XO males, phenotypically *y w sn* and are thus easily distinguishable from the rest of the offspring. As XO males are due to losses of X- or Y-chromosomes both females and males are counted and the rate of XO males per total offspring is calculated. In this experiment only two series were carried out, series M and series iF-M.

The result (table 5) is significantly in favour of the hypothesis of an increased rate of breaks induced in spermatozoa irradiated in impregnated females as compared with males.

4. COMPARISON OF FULL YELLOW MUTATIONS AND HYPERPLOID MALES IN THE PRECEDING EXPERIMENTS

In the first four groups of experiments presented it has been shown that when spermatozoa were irradiated in impregnated females a higher rate of rearrangements was induced than when irradiated in the males. When comparing the rates of hyperploid males one sees that differences exist between the various types of mating with each mode of irradiation. In order to facilitate a comparison the rates of hyperploid males and *yellow* mutations (in the M_5 chromosome) are combined in table 6.

TABLE 5

Offspring from crosses of y w sn ♀♀ × M5 ; sc^8 Y ♂♂. *The females were guaranteed to be free from extra Y-chromosomes by a special technique described in the text. Both females and males were mated at an age of 2-4 days. The two series M and iF-M were carried out parallelly. Elimination of the paternal X- or Y-chromosome gave rise to XO males, phenotypically* y w sn, *distinguishable from the normal XY males, which were phenotypically* w sn *as the Y chromosome is a* sc^8 Y, *containing the normal* yellow *allele. Both females and males were counted as XO males were result of elimination of X- or Y-chromosomes.*

Series	Total males XY and XO	Total females	Total offspring	XO males	
				abs. nr.	Per cent.
M . .	4,774	5,226	10,000	82	0·82
iF-M . .	2,168	2,533	4,701	62	1·32

First studying the rates of full *yellow* mutations induced in M_5 chromosomes in the two types of matings a difference is observed in series M only where a χ^2_c test gives $0{\cdot}02 > P > 0{\cdot}01$. In the other two series the rates are about the same when comparing within each series. The high rate of *yellow* mutations in series M when *y ac sc pn sn* females were mothers has no correspondence in the rate of hyperploid males when compared to the rates in series vF-M. It is not possible at present to explain this discrepancy.

The rates of hyperploid males will now be compared :

1. Comparing the rate of hyperploid males induced in M_5 chromosomes versus in Canton S X-chromosomes in the offspring from *y w sn* females there is a significantly higher rate in the M_5 chromosome than in the Canton S X-chromosome (series M and vF-M).

2. The same comparison as in 1, but in the offspring from *y ac sc pn sn* females give the same significant result as in 1 (series M).

3. Comparing the rates of hyperploid males induced in Canton S X-chromosomes in the offspring from *y w sn* females versus from *y ac sc pn sn* females there is a significantly higher rate in the latter than in the former (series M).

4. The same comparison as in 3 but in M_5 chromosomes gives a significantly higher rate of hyperploid males in the offspring from *y ac sc pn sn* females than from the *y w sn* females (series M and vF-M).

The differences in the rates of hyperploid males in the offspring from *y ac sc pn sn* females versus *y w sn* females are independent of irradiation of the females in some series. We may thus conclude that there are some kind of differences between the two types of females used as regards the probability of occurrence of hyperploid males, deficient for nearly the whole paternal X-chromosome.

In some earlier experiments (Lüning, 1952*a*) a difference in the

TABLE 6

The rates of hyperploid males from tables 1-4 and of yellow mutations from tables 1 and 4

Series	Hyperploid males				Yellow mutations	
	Canton S ♂♂		M_5 ♂♂		M_5 ♂♂	
	y w sn ♀♀	*y ac sc pn sn* ♀♀	*y w sn* ♀♀	*y ac sc pn sn* ♀♀	*y w sn* ♀♀	*y ac sc pn sn* ♀♀
M . .	6	10	10	21	19	29
vF-M . .	6	...	9	21	23	24
iF-M . .	13	21	20	29	28	29

Comparing *yellow* mutations in series M . . . $\chi^2_c =$ 5·6 0·02>P>0·01

Comparing hyperploid males :

Series M+vF-M with *y w sn* mothers ; Canton S *vs.* M_5 $\chi^2_c =$ 5·5 0·02>P>0·01
Series M with *y ac sc pn sn* mothers : Canton S *vs.* M_5 . $\chi^2_c =$ 9·5 0·01>P>0·001
Series M with Canton S fathers : *y w sn* vs. *y ac sc pn sn* . $\chi^2_c =$ 3·5 0·05>P>0·02
Series M+vF-M with M_5 fathers : *y w sn* vs. *y ac sc pn sn* $\chi^2_c =$ 30·6 P≪0·001

rates of hyperploid males was used as indicating a difference in the rates of breaks. By analogy we may suggest that the rates of breaks, available for rearrangements, might be influenced in some way by the eggplasm. If this was true we would expect a corresponding increase in the rates of *yellow* mutations in the M_5 chromosome. Of the three series it was shown above that there is a true difference in one only. In all series taken together there is not a significant difference, χ^2 test gives 0·10>P>0·05.

Another fact that speaks against the present hypothesis is the following : Bonnier and Lüning (1951) demonstrated that the rate of hyperploid males was proportional to $(\text{dose})^{1.5}$. If now the

proportion of broken ends taking part in rearrangements is higher when in *y ac sc pn sn* eggs than in *y w sn* eggs one would *a priori* expect that the increase in the rate of breaks in series iF-M versus series M and vF-M would more strongly increase the rate of hyperploid males in experiments with *y ac sc pn sn* mothers than in those with *y w sn* mothers. This is not the case as is seen from comparisons of the ratio of hyperploid males in series iF-M versus series M and vF-M in the four groups of experiments presented in table 6. There was instead a lower increase when M_5 males were used. Hence it is not probable that the differences in the rates of hyperploid males are due to differences in the ratio of broken ends which take part in rearrangements or restitute in the original order.

As the differences between the rates of hyperploid males in the various groups of experiments do not seem to be due to the influence of the mother's genotype (nuclear or plasmic) on the proportion of recombinations in the paternal X-chromosome we may suggest that the differences under consideration are due to differential viability of the potential hyperploid males. It was supposed (Lüning, 1952*a*) that most hyperploid males occur from two breaks in the X-chromosome, one in the distal and the other in the proximal region. The differences between the rates of hyperploid males could then be explained either by supposing that the viability of cases with a longer distal part is higher in *y ac sc pn sn* eggs than in *y w sn* eggs or that it is differences in the proximal (heterochromatic) regions in *y ac sc pn sn* and *y w sn* chromosomes or a combination of both possibilities. Comparing the rates of hyperploid males in tables 2 and 3 one sees that in table 3 the rate of cases with breaks to the left of *pn* is higher than the total rate in table 2. The rate of cases with breaks to the left of *pn* is low. We may thus conclude that the difference between the rates of hyperploid males from *y ac sc pn sn* versus *y w sn* mothers are not due to differences in the length of the distal region of the paternal X-chromosome included in these males. The possibility then remains that it is due to the heterochromatic region. In favour of this suggestion is the fact that in M_5 chromosomes, with both distal and proximal heterochromatic regions, a higher rate of hyperploid males is induced than in Canton S X-chromosomes. It is possible to test the influence of the proximal heterochromatic region but such an investigation is beyond our present programme.

5. DISCUSSION

Herskowitz (1951) suggested three different origins of recessive lethals: "point mutations, independent of breakage; breakage alone; and position effect following rearrangement of breaks". I showed (1952*b*) that the rates of recessive lethals induced in spermatozoa irradiated at different stages of spermiogenesis varied but not as much as the rates of breaks. For that reason I concluded that recessive lethals are due to both *intra-* and *inter*genic changes. My

term *intra*genic changes is nearest comparable to Herskowitz's " point mutations, independent of breakage ". The proportion of recessive lethals of this origin seems, however, to be higher in my material than in Herskowitz's. This discrepancy will be dealt with in a future paper.

At present we are interested in the fact that recessive lethals occur in two ways, *intra-* and *inter*genic changes, and that the proportion of them may vary. It means that a change in the rate of recessive lethals may be due to an increase in *intra-* and/or *inter*genic changes. Bonnier and Lüning (1953) showed that when spermatozoa were irradiated in impregnated females there was induced a higher rate of recessive lethals than when irradiated in males. It was consequently of interest to find out if this increase in recessive lethals was due to an increase in *intra-* or in *inter*genic changes or in both. In the preceding paragraphs it was shown that the rate of rearrangements was higher in spermatozoa irradiated in impregnated females as compared with males. It was concluded that this was due to an increased rate of breaks rather than to a different rejoinability of broken ends.

The present analysis of chromosome aberrations was based on three different types, viz. hyperploid males, *yellow* mutations in the M_5 chromosome (minute rearrangements) and X- and Y-chromosome losses. The rate of hyperploid males has been shown (Bonnier and Lüning, 1951) to increase in proportion to $(\text{dose})^{1.5}$, *i.e.* $(\text{rate of breaks})^{1.5}$. Hence it is an agreement with expectation when it is found that the rate of hyperploid males increases more than the rates of *yellow* mutations and X- and Y-losses, which increase linearly with the dose according to Belgovsky, (1939) and Muller, (1940). A calculation of the increase in the rate of breaks based on a comparison between the different types of aberrations would be impaired by great uncertainties. We may, however, suggest that the true increase in the rate of breaks lies between 25 and 60 per cent. These two figures are taken from *yellow* mutations and X- and Y-chromosome losses. The corresponding variation in the rates of recessive lethals lies between 10 and 25 per cent., only Gulbekian found 70 per cent. (Bonnier and Lüning, 1953). Hence the observed increase in the rate of breaks seems to be greater than that of recessive lethals. Since recessive lethals are partly dependent and partly independent of breaks we must conclude that the increase in the rate of recessive lethals is mostly due to *inter*genic changes. From this comparison it is impossible to exclude a minor increase in the rate of *intra*genic changes as well. There are, however, two sets of experiments above in which changes, supposed to be *intra*genic, have been studied, viz. *yellow* mutations in the wild type X-chromosome. There was no indication of an increased rate of these changes. Taken together the two results point in the same direction, that there is no, or only a very small, increase in *intra*genic changes.

The cause of the increased rate of breaks is, however, still obscure. Several possible explanations have been discussed by Dr Bonnier in his paper. Only one further possibility will here be put forth : and this is not an explanation, it is merely putting the problem off to a point beyond which we cannot reach—at present. I demonstrated (1952*b*) that the rate of breaks varied with the stages of spermiogenesis treated, as well as with the age of males at treatment. The variation of the breakability with the age of males in the mature spermatozoa was not synchronised with its variation in the inverse direction in the more sensitive stages. Changes of the physiological conditions in males when ageing seemed thus to influence the various stages of spermiogenesis in a different manner. Hence perhaps the new physiological environment—the female receptacles—changes the breakability of the spermatozoan chromosomes in a similar manner. The nature of these changes is, however, still hidden. The present investigation has at least shown that the variation in sensitivity may occur in both directions : In spermiogenesis it is lowered and in impregnated females it is raised.

6. SUMMARY

1. The present investigation deals with an analysis of the rates of rearrangements induced in sperm of *Drosophila melanogaster* when irradiated in females as compared with in males.

2. There were induced significantly more hyperploid males when irradiated in impregnated females than in males. The rates of *yellow* mutations in the M_5 chromosome pointed in the same directon but not with the same strong significance.

3. The rate of hyperploid males is also dependent on both the paternal and maternal stocks. A possible cause of this is discussed.

4. It is concluded that more chromosome breaks are induced in spermatozoa irradiated in impregnated females than in males, and a possible cause of this is discussed.

Acknowledgment.—This investigation was supported by the Swedish Natural Science Research Council.

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NEW EVIDENCE ON THE ORIGIN OF THE OLD WORLD COTTONS

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1. INTRODUCTION

In the *Evolution of Gossypium* (Hutchinson, Silow and Stephens, 1947) it was suggested that the true cottons owe to human agencies their separation from the nearest wild species, their specific differentiation, and their striking evolutionary success. It followed that the whole of the diversification of the lint bearing species of *Gossypium* must be regarded as having taken place since the dawn of civilisation, and that the modern cottons represent the product of an extremely rapid evolutionary process. Further progress has now been made in the elucidation of the relationships of the Old World cottons (Hutchinson, 1949, 1950), and of their wild relatives (Knight, 1949; Douwes, 1951; Douwes and Cuany, 1951, and 1952), and a fresh assessment of the evidence on the origin and antiquity of the diploid cottons is therefore appropriate.

2. THE RELATIONSHIP OF THE DIPLOID COTTONS TO THE WILD SPECIES

There are two groups of wild species of *Gossypium* native to Africa and Asia, the Anomala and the Stocksiana. Since the Old World cottons are fairly closely related to the former, and only very distantly to the latter, an understanding of the relationships of the wild species is a prerequisite to a discussion of the origin of the cottons. Recently Douwes (1951) has shown that *G. somalense* belongs in the Stocksiana, and Knight (1949) has recorded that it is distributed from northern Kenya to British Somaliland, and across the northern Sudan as far west as Ennedi in the eastern districts of French Equatorial Africa. Douwes and Cuany (1952) have shown that *G. areysianum*, formerly included in the Anomala on the original somewhat cursory description, also belongs morphologically and cytogenetically in the Stocksiana. Further, they have shown that *G. triphyllum* belongs in the Anomala, and the Anomala can now be defined with confidence as including *G. anomalum* and *G. triphyllum* only. Both species occur in arid regions in southern Angola and South-West Africa, and *G. anomalum* is distributed across the southern Sahara from the French Sudan to the upper reaches of the valley of the Baraka river in Eritrea. The distribution of these species is mapped in fig. 1.

Hutchinson, Silow and Stephens (1947) suggested that the Old

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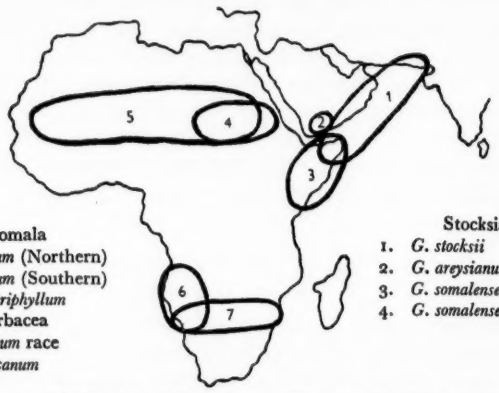
World cottons of the Section Herbacea arose under cultivation in Sind. Believing at that time that the Arabian *G. areysianum* belonged in the Anomala, they supposed that the original range of the Section included southern Arabia and the Red Sea region, and that the material first cultivated in Sind might have been collected by travellers to Arabia and neighbouring regions. It is now evident that the Arabian and Red Sea region is the territory of the Stocksiana and not of the Anomala, and the source of any parent type related to the Anomala must be sought far to the west or south west. The hypothesis that the prototype of the Herbacea existed in the Red Sea and Arabian region thus becomes untenable.

Cytologically the A genom of the Asiatic cottons and the B of the Anomala are the two most closely related of the genoms into which Beasley (1942) divided the genus *Gossypium*. In fact, but for



Anomala
5. *G. anomalum* (Northern)
6. *G. anomalum* (Southern) and *G. triphyllum*

Herbacea
7. *G. herbaceum* race *africanum*

Stocksiana
1. *G. stocksii*
2. *G. areysianum*
3. *G. somalense* (Somalia)
4. *G. somalense* (Sudan)

FIG. 1.—Distribution of Wild Species of *Gossypium* in Africa and Asia.

their radically different evolutionary status, they might have been regarded as scarcely sufficiently widely separated to justify equal rank with Beasley's other groups. In considering the genesis of the Herbacea, therefore, it is necessary to ignore the vast diversity of the cottons and the great extension of their range under cultivation in recent times, and try to reconstruct the status and distribution of the species of the A/B genoms before the intervention of man.

Considering the wild species of *Gossypium* only, there is good evidence for allopatric origin throughout the genus. Many species have distributions that do not overlap at all, and where overlapping occurs, it would be eliminated by a moderate contraction of the area now occupied. So it may be supposed that the first A bearing species was probably allopatric in relation to the early B species, and arose in some area adjacent to, but not within, that occupied by the early representatives of the Anomala. Since *Gossypium* is a genus of

desert plants, this original area must have been in arid country. In so far as the distribution of the modern Anomala is a guide, there are two arid areas that may be considered. These are the Sudan-Somaliland region in the north, and the dry bushveld area stretching from Ngamiland to southern Portuguese East Africa in the south. In the former, there is nothing other than *G. anomalum* related to the Asiatic cottons, but two species of the Stocksiana are indigenous. It is, therefore, unlikely that the prototype of the true cottons was to be found there. The latter, on the other hand, though not occupied by any of the lintless species of *Gossypium*, is the habitat of a true cotton, *Gossypium herbaceum*, race *africanum* (see fig. 1). This cotton is a perennial shrub without the distinguishing characters of the modern agricultural races of the species, and is to be found growing wild in natural vegetation. Reasons have been given, however, (Hutchinson, Silow and Stephens, 1947; Hutchinson, 1949) for believing that it is a wild derivative of a cultivated race, and the evidence must be re-examined in detail before it can be considered as a possible primitive type close to the ancestral form of the Old World cottons.

With the exception of *africanum*, the introduction of all the cottons now to be found in Africa can be traced with some confidence. The account (Hutchinson, 1949) of those of northern Africa requires no modification. On the cottons of eastern and southern Africa, some further information is now available. A description of the coast of East Africa in 1516 A.D. by Duarte Barbosa (Hakluyt Society, 1866) includes an account of the use of cotton in the region of Sofala. According to Barbosa, the "Moors" of Sofala traded cotton goods and some silk, obtained from Cambay (in Western India) by way of the East African ports of Malindi, Mombasa and Kilwa, for gold from the kingdom of Monomotapa. He continues,

> "The Moors have now recently begun to produce much fine cotton in this country, and they weave it into white stuff because they do not know how to dye it, or because they have not got any colours . . ."

This account of the initiation of cotton cultivation on the East African coast fits in with the known distribution of *G. arboreum* race *indicum*. It has been pointed out (Hutchinson, 1950) that the *indicum* cottons of the East African coast and Madagascar resemble the *Rozi* cottons of Western India, as would be expected if they were introduced from the kingdom of Cambay. Moreover, they are still to be found on the coasts and islands of East Africa as far north as Somalia, which is in agreement with Barbosa's statement that the trade route was by way of Malindi, Mombasa, and Kilwa. Thus cotton spinning on the East African coast can be regarded as a comparatively recently introduced craft, associated in both origin and distribution with *G. arboreum* race *indicum* from Western India, and not with *G. herbaceum* race *africanum*.

The *africanum* cottons have also been further studied recently.

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Quintanilha *et al.* (1948) found the northern limit of the race in Portuguese territory was the Save River. It is to be found further north on the Rhodesian side of the border, and Quintanilha has recently (in correspondence) recorded it in Portuguese territory as far north as the Buzi River, and (as a single plant) on the right bank of the Zambesi at Carinde. In all cases it was found in open savannah forest and grassland associations. This area covers the localities from which herbarium material has been collected, and obviates the argument (Hutchinson, 1949) that *africanum* has suffered in recent times a diminution of area to be ascribed to the abandonment of cultivation. Moreover, Mr Gomes Pedro has pointed out (in correspondence) that " if it is really an introduced plant " it is surprising that " it disappeared almost completely along the known roads of penetration of pre-European merchants in spite of the fact that apparently the same ecological conditions as in Sul do Save are to be found in some patches along these roads." This is a particularly relevant comment, since elsewhere in Africa where cultivated cottons have run wild they are to be found on field margins, in house yards, and in the secondary associations that develop when natural vegetation is disturbed by man. It thus appears that the argument that *africanum* is a wild derivative of a cultivated cotton can no longer be sustained.

The evidence of the bollworms

Positive evidence that *africanum* is indigenous in southern Africa has recently been provided by studies of the taxonomy of the Red and Sudan bollworms. The Red bollworm pest was first described as *Diparopsis castanea* on a specimen from Delagoa Bay. It is known from Portuguese East Africa and neighbouring regions of South Africa, Rhodesia and Nyasaland. Recently, following the extension of cotton growing in Portuguese East Africa, it has appeared in the Rovuma river valley, and has gained a foothold in southern Tanganyika. What was believed to be the same insect is a well-known pest of cotton across the savannah belt of West Africa and in the Sudan, where it is known as Sudan bollworm. Since these bollworms are confined to cotton, and to the South African *Cienfugosia hildebrandtii*, their distributions provide information on the original distribution of their host plants.

Recently the classification of *Diparopsis* has been re-examined (Clements, 1951 ; Pearson, 1954), and four species recognised. Of these the cotton pest of the savannah regions of West Africa and the Sudan is assigned to *D. watersi*, accessions from Angola to *D. tephragramma*, and moths bred from larvæ found on *Gossypioides kirkii* in Tanganyika are named *D. gossypioides*. *D. castanea* proper is the Red bollworm of South Africa, Portuguese East Africa and Nyasaland.

From the point of view of the student of evolution it is unfortunate that work on *Diparopsis* has been so largely confined to studies of its

occurrence on cultivated cottons. The host range has, however, been worked out for *D. castanea* (Marshall, Parsons and Hutchinson, 1937), and it is known that this species is confined to cultivated cottons, spontaneous *G. herbaceum* race *africanum*, and *Cienfugosia hildebrandtii*. The cultivated cottons are introductions of the last four centuries, and therefore represent recent additions to the host range. *Diparopsis* is not found in the northern part of the range of *Cienfugosia hildebrandtii* but only where the distribution of the *Cienfugosia* overlaps that of the *africanum* cottons. It may be concluded, therefore, that the *Cienfugosia* is not a primary host.

Of the other three species, *D. gossypioides* is only known from a few specimens collected from *Gossypioides kirkii*. *D. tephragramma* has been collected from cultivated cotton in Angola (Pearson, personal communication), and *D. watersi* is a pest of cultivated cotton in the West African and Sudan region. In the area of all species cotton is introduced and, as in southern Africa, must be regarded as a relatively recent addition to the host range of *Diparopsis*. Unfortunately, there is no direct evidence as to the original hosts of *D. tephragramma* and *D. watersi*. It seems probable that *D. tephragramma* depended on *Gossypium triphyllum* or *G. anomalum*, as these two species are indigenous in the area in which *D. tephragramma* occurs.* Similarly, *D. watersi* may be supposed to have lived on *G. anomalum* in the northern part of the range of the latter. Observations on *G. anomalum* in the northern Sudan indicate that it is attacked by bollworms in the wild as well as in experimental plots, but no opportunity has arisen of searching for Sudan bollworm on colonies of *G. anomalum* growing in areas remote from commercial cotton cultivation.

The three species of *Diparopsis* that attack cultivated cottons are all to be found in similar ecological zones, and the habits and life histories of *D. perditor* and *D. castanea* (the only two that have been adequately studied) are virtually the same (Geering and Baillie, in press). Only in terms of isolation on three distinct hosts can the emergence of three distinct species of *Diparopsis* be understood. It follows that a distinct host plant must have existed in the area originally occupied by *D. castanea* for a period long enough for the evolution of the species. This can only have been *G. herbaceum* race *africanum*, and race *africanum* must, therefore, be an ancient truly wild plant, and not a recent escape from cultivation.

3. THE ORIGIN OF THE CULTIVATED DIPLOID COTTONS

Having given reasons for believing that one diploid cotton is truly wild, it becomes necessary to re-examine the possibility that others may be also. In *G. herbaceum* there are no other forms concerning

* A Red bollworm egg was observed on a herbarium specimen of *G. triphyllum* (Parnell, personal communication) some years before it was found that there was more than one species of *Diparopsis*.

which there can be any doubt. Chevalier (1936) and Hutchinson (1949) have shown that the north African *G. herbaceum* race *acerifolium* is never found beyond the shelter afforded, directly or indirectly, by man's agricultural activities. Data on recent collections and herbarium material from Arabia indicate that race *acerifolium* is an associate of man there also. The other races of the species have only been recorded in cultivation.

Forms of *G. arboreum* have been recorded growing spontaneously in many areas, and the possibility that the species was independently domesticated from the wild requires careful consideration. The most important collections of spontaneous *arboreum* cottons are those from Western India and the Deccan discussed by Watt (1907). In considering his views it must be remembered that he considered it natural that wild cottons should be found alongside cultivated forms. In discussing *G. arboreum*, he stated firstly that

> " It is doubtful whether any botanist has ever discovered it in what could be regarded as an undoubted indigenous habitat in any country ", and then went on to say : " Thus while little is known for certain of the origin of *Gossypium arboreum* there seems no doubt whatever that it played an important *role* in the production of some of the best-known cultivated cottons of the Old World. It is accordingly desirable to assume the existence of a wild species, such as that briefly indicated by the older authors. And in part support of that view it may be added that when neglected, the cultivated forms become perennials and rapidly assume the leading characteristics of the plant briefly defined above, . . . "

When such views obtained there could be no recognition of the need to distinguish critically between plants occurring more or less spontaneously in secondary man-made habitats, and those having an established place in undisturbed indigenous vegetation. Watt (1907) collected cottons growing spontaneously in Kathiawar, Gujerat, Khandesh, and the Deccan. He stated,

> " I have in India repeatedly collected a wild or self-sown *Gossypium*. . . It literally envelops the old fort of Junagardh and the subterranean Buddhist palaces of that neighbourhood. It is common on the sacred Jaina hill of Palitana growing among the indigenous scrubby vegetation where, for some centuries possibly, there has been no cultivation. It is fairly common in the hedges of Gujerat . . . and was found by me in Khandesh and in the Deccan, especially near the Buddhist cave temples of Elura and Ajunta. If in all these instances it has to be regarded as but a survival of former cultivation, there would seem every likelihood that in some of its known habitats it has existed in that condition for a great many years, perhaps centuries."

Of *Rozi*, the cultivated form of this perennial, Watt stated, " When left to grow in the hedgerows *rozi* becomes subscandent, the wool shortens and turns rufous-coloured, and the fuzz lengthens and also becomes red-coloured." Evidently there is no sharp distinction between the wild growing and cultivated forms, and the habitats of those found wild are all on cultivation margins or on the abandoned sites of human activity. More recently, Chevalier (1936) and Hutchinson (1949) have examined the status of *G. arboreum* race *soudanense* in the field in West Africa and the Sudan, and concluded that it is entirely

dependent on cultivation or on the secondary habitats opened up thereby. There is thus a close parallel between the habits of the Indian and African forms of *G. arboreum*, and it may be concluded that in both regions the species is always a cultigen or is derived from a cultigen.

Cottons of the species *G. arboreum* have been reported as wild in the Indonesian archipelago and the Philippines. Herbarium material of most of the morphologically distinct forms of *G. arboreum* is available from Indonesia, and much of it is known to have been cultivated. The only living material that has been examined consists of one perennial form from the Philippines, said to be wild, and two accessions of a cultivated annual known as Demak from Java. Types that were probably perennial are represented by specimens from Ticao in the Philippines, British North Borneo, Gorontalo and Macassar in the Celebes, and the islands of Leti, Lakor and Timor. Probable annuals, mostly known to be cultivated, have been collected in Culion and Panay in the Philippines, and from a number of places in Java. Evidently cottons have been sufficiently widely spread and used in the area to account for the presence of forms that have run wild. Though direct evidence of the dependence of such types upon man is lacking, the possibility that they might be truly wild and endemic can be ruled out on ecological grounds. It has been shown repeatedly (Hutchinson, Silow, and Stephens, 1947; Hutchinson, 1943; Hutchinson, 1944) in various parts of the world that the genus *Gossypium* is xerophytic, and that even its most mesophytic members are intolerant of competition, particularly in the seedling stage. In southern Africa *G. herbaceum* race *africanum* is to be found in open vegetation in areas with rarely more than 30 inches of rain. In western India *G. arboreum* race *indicum* has established itself in regions of similar rainfall and correspondingly open vegetation. In the Indonesian and Philippine region, areas with a dry season are limited, and even these rarely receive less than 40 inches of rain per annum. To quote Braak (1945), "A yearly amount of less than 1000 mm. is quite exceptional", and has in fact only been recorded at four rainfall stations in Indonesia. In such conditions the height and density of natural climax vegetation would preclude the establishment of any member of the genus *Gossypium*, and it may be concluded that the cottons were introduced by man and are maintained, directly or indirectly, by his activities.

G. arboreum has been recorded as wild in Madagascar. From herbarium specimens and living material grown from Madagascar seed, the type can be assigned with confidence to *G. arboreum* race *indicum*.* It is in fact indistinguishable from perennial forms from western India. In parts of the island it has been maintained in house yards and even cultivated until recent times. Plants were raised

* *G. herbaceum* L. var. *perrieri* Hoch. and *G. mahafalense* have been used for Madagascar collections.

from two accessions of seed. They were tall, late fruiting perennials, and were very similar in appearance, except that the one recorded as wild was later fruiting. There was obviously a very close relationship between the two, and the question is simply whether the wild is derived from the cultivated or *vice versa*.

M. Perrier de la Bathie has summarised in correspondence existing knowledge of the status of *G. arboreum* in Madagascar. He pointed out that there are in north Madagascar remains of ancient (but undated) Hindu-Arab settlements, and in west Madagascar traces of an even older Bantu colonisation. *G. arboreum* is found throughout the length of the west coast in three forms, which he described as follows (trans. from French) :

" 1. primitive form, arborescent, which no longer exists except in the xerophytic woodlands of the S.W. ;

" 2. ruderal form, perennial, ± planted by the natives in the vicinity of their villages, probably relics of ancient cultures ; and

" 3. cultivated form, herbaceous, a culture which was still practised in 1900-12, and which may or may not continue at the present time." *

The distribution of the three forms is given in the map in fig. 2. It will be seen that the cultivation relic form extends throughout the area of the two ancient colonisations, while both the cultivated form and the " primitive form " now only found wild, are found in the southwest. The limitation of the " primitive form " to xerophytic woodlands is as would be expected. It is only in such habitats that *Gossypium* can maintain itself without the intervention of man. The status of *G. arboreum* in Madagascar is, therefore, consistent with the theory that it was introduced into the island as a cultivated plant, perhaps by the Hindu-Arab community, and became established as a secondarily wild plant in the xerophytic vegetation in the southwest of the island. The alternative, that the wild *arboreum* of southern Madagascar is the primitive form of the species, cannot be ruled out on present evidence. That *G. arboreum* originated in the wild just across the sea from the endemic area of *G. herbaceum* race *africanum* is an attractive hypothesis, and a close study of the species in Madagascar with this possibility in mind would be instructive. Nevertheless, the genetic evidence is in conformity with that adduced above from morphological and geographical considerations in supporting the view that *G. arboreum* in Madagascar was derived from Indian forms, and not the reverse. *G. arboreum* and *G. herbaceum* are so closely allied as to give vigorous, fully fertile F_1s, and the species barrier only acts through genetic breakdown in later generations. It will be shown below that the closest relationship is between the *arboreums* of western India and *G. herbaceum* race *acerifolium* from Arabia, and not the wild *africanum* from southern Africa, and it will be suggested that between the Arabian and Indian forms the species barrier may well prove to be of little account. The weight of evidence is therefore in

* Among accessions from Madagascar grown since this was written, the annual form proved to be *G. hirsutum* race *punctatum*.

favour of the view that *G. arboreum* was never truly wild, but arose in cultivation by differentiation from a cultivated stock of *G. herbaceum*.

The development of the cultivated races of *G. herbaceum* took place

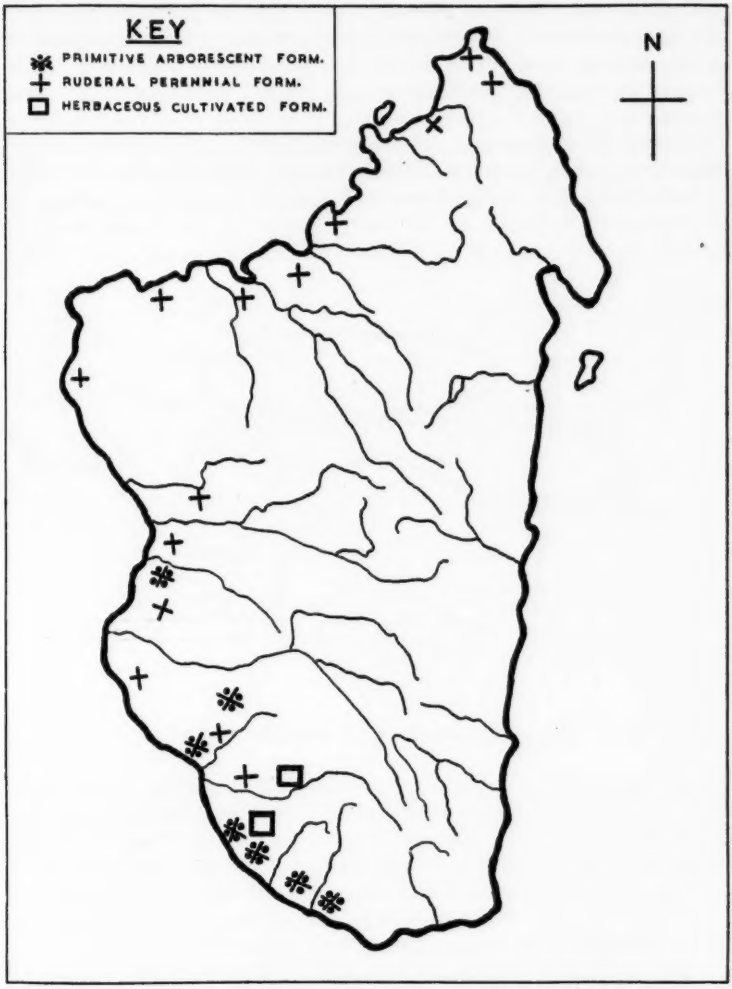

FIG. 2.—Outline map of Madagascar showing records of *G. arboreum* race *indicum* after M. Perrier de la Bathie.

far to the north of the area in which the wild *G. herbaceum* race *africanum* is now to be found. (see distribution map, fig. 3.) There seems no reason to suppose that the range of *africanum* was ever so extended,

and there is no evidence that any form of *G. herbaceum* was wild in any of the territories in which it is now either cultivated or commensal. It seems probable, therefore, that the wild plant was introduced into the country in which it was first domesticated. Trade between India and Arabia and the East African coast is very ancient and gold from the region in which *G. herbaceum* race *africanum* is wild was important in it. It may well be, therefore, that wild cotton was taken home by traders from the north who were primarily interested in other things.

It has been suggested (Hutchinson, Silow and Stephens, 1947) that the cultivated cottons arose in the lower Indus valley, in what is now Sind. The native cottons of Sind in recent times belong to *G. arboreum*. Evidence on the early cottons of the Indus valley is limited to that gleaned by Gulati and Turner (1928) from three

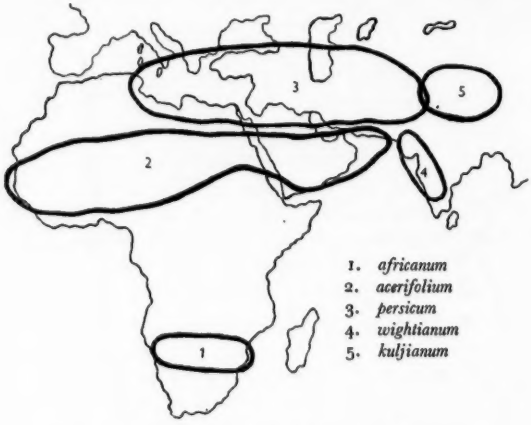

FIG. 3.—Distribution of Races of *Gossypium herbaceum*.

fragments of cotton materials recovered from the ruins of Mohenjo Daro and dated *c.* 3000-2750 B.C. They concluded that the lint hair characters of the ancient cotton resembled those of coarse *arboreum* cottons of the present day, and not those of modern Indian *herbaceums*. If the original lint bearing cotton was a wild type of which *G. herbaceum* race *africanum* is the modern representative, the theory that domestication took place in Sind is untenable.

The earliest cottons must be sought within the present range of *G. herbaceum*. The most primitive of the cultivated *herbaceums* are the perennials related to race *acerifolium*. Cottons of this type are to be found all the way from the Mekran in Baluchistan (Ansari, 1941) through Arabia, and across the Sahara to West Africa. They appear to have been introduced into Africa by the Moslems (Hutchinson, 1949), and their original home may be taken to be Arabia or the

coasts of Persia and Baluchistan. Thus the most primitive cultivated *herbaceums* are to be found on the coasts bordering the great Indian Ocean trade route by which it must be supposed that the introduction of their wild ancestor from southern Africa was effected. From thence, the differentiation of the *herbaceum* cottons took place under the selective pressure of new environments as the new crop spread. In Persia, the annual race *persicum* was developed to meet the limitations imposed by cold winters, and further north the very short term race *kuljianum* arose in response to selection in an area of short, hot summers and long, cold winters. Later, when the advantages of the annual habit became apparent in India, annual *herbaceums* were carried south to replace perennial *arboreums* and gave rise to race *wightianum* in western India.

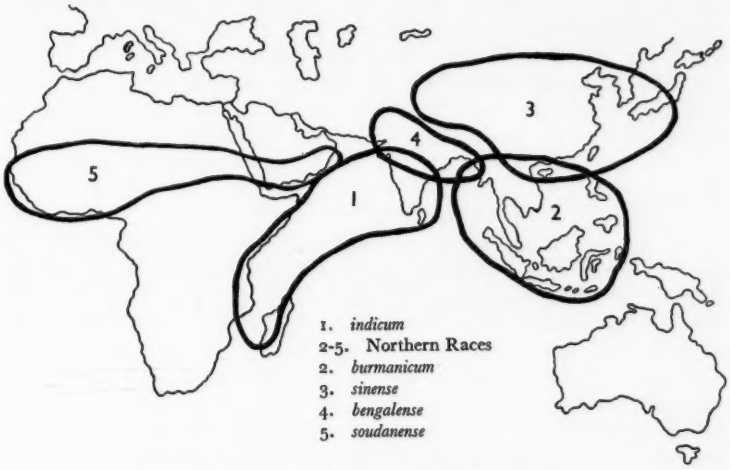

FIG. 4.—Distribution of Races of *Gossypium arboreum*.

In considering the origin and development of *G. arboreum*, the species may be divided into two parts. On the one hand are the *indicum* cottons of peninsular India. Among these are to be found both the primitive perennial forms that were collected in wild secondary vegetation by Watt, and also the annual high quality cottons of the crops of the Deccan and Madras. On the other hand are the *burmanicum* cottons of Assam and Burma, and related perennials to be found occasionally throughout the Ganges valley and neighbouring regions, Rajputana, the southern Punjab, and Sind. From this perennial group arose two great annual races, the *bengalense* cottons of the commercial crops of northern India and the *sinense* cottons of China (see distribution map, fig. 4).

Silow (1944) showed that genetically race *indicum* is more closely related to *G. herbaceum* than are the other races of *G. arboreum*. This

conclusion is supported by morphological data. Watt (1907) classified the perennial forms of *G. arboreum* race *indicum* as *G. obtusifolium*, and made *wightianum* a variety of it. There can be no doubt now that the perennial *indicums* belong in *G. arboreum*, and the annual *wightianums* in *G. herbaceum*, but they are nevertheless not very widely distinct. Even closer morphologically are the perennials of *G. herbaceum* race *acerifolium* from Arabia and the perennials of *G. arboreum* race *indicum* from western India. Indeed, in the absence of fruiting material it is often impossible to say with confidence whether an Arabian specimen of this type belongs in *G. arboreum* or *G. herbaceum*. On any theory of the differentiation of *G. arboreum* and *G. herbaceum*, these two must be regarded as the types linking the species.

These perennial *indicums* are to be found in profusion in Kathiawar and Gujerat in Western India. Southwards throughout peninsular India primitive forms are occasional, and more advanced perennial forms not uncommon, while the great commercial crops are planted with their annual derivatives. From the distribution of the more primitive types it is clear that the centre of the race is in Kathiawar and Gujerat, and it has spread thence into peninsular India. Cambay is in the ancient home of the race, and from Cambay it spread also along the Indian Ocean trade route to the East African coast and, it is suggested, also to Madagascar.

G. arboreum race *indicum* is morphologically rather uniform. It tends to form rounded bushes when well grown, with broad leaves, little or no anthocyanin pigment, and yellow flowers. The perennial forms of *G. arboreum* from the Indo-Gangetic plain, and those of *G. arboreum* race *burmanicum* in Assam and Burma, are pyramidal shrubs with long flexible branches, and are highly variable in leaf shape, anthocyanin pigmentation, and flower colour. Because of the inadequacy of the available material, Silow was unable to assess the position of the north African *G. arboreum* race *soudanense*. Extensive further collections have been made, and it is now clear that the *soudanense* cottons are pyramidal shrubs with long flexible branches, and are, therefore, related to the *arboreum* cottons of northern India rather than those of race *indicum*. In addition they exhibit the variability in minor characters typical of the northern group.

Gulati and Turner's (1928) studies showed that in measurable characters the Mohenjo Daro cotton resembled modern cottons of the northern group rather than the *indicums*, and it may be suggested that the part played by the Indus civilisation in the rise of the cotton crop, which was undoubtedly an important one, was the development and spread of the northern *arboreums*. This group is the real backbone of the Old World cotton crop. Westward it gave the first cottons of Africa, first at Meroe on the Nile and later right across the savannahs of the Sudan to West Africa. Eastward it gave the cottons of the Ganges valley, Assam, and Burma, and thence gave rise to the two great annual races, *bengalense* in northern India, and

sinense in China. Moreover, it appears that the cottons of Indonesia and the Philippines are of northern, and not peninsular, Indian origin. The only perennial form that has been grown in culture was of the pyramidal habit, and records of narrow leaf and red flower in Indonesian cottons are common in the literature. Perennials are well known in Burma, where they still contribute to the commercial crop, and they have also been collected in Siam. A careful study of Watt's quotations of specimens, however, reveals none from Malaya. Moreover, in both Indonesia and the Philippines cotton is known as "*kapas*" or "*gapas*" which is the northern Indian name for seed cotton (Sanskrit, Hindustani, Bengali, and related languages). Hence it is likely that the early northern cottons spread south eastward from Burma to Indonesia through Siam.

There is thus evidence of three waves of development in the Asiatic cottons. First came the evolution of domesticated forms of *G. herbaceum* in Arabia and Persia. Then followed the differentiation of *G. arboreum* race *indicum* in western India, and finally the northern *arboreums* of the *burmanicum-soudanense* group were evolved in the Indus region. Subsequent development went on in parallel in the three groups. Improvement in agricultural characters, the evolution of the annual habit, and the development of resistance to such diseases and pests as bacterial blight and jassid, can be seen in all three. Nevertheless the three stocks were not equally successful. The most recent has covered the greatest area, stretching from the Atlantic in West Africa to the Pacific in Japan, and has developed the largest crop populations, with the great bulk of the Chinese crop and of the crops of northern and central India to its credit. *G. arboreum* race *indicum*, which is more recent than *G. herbaceum*, has surpassed the older species in agricultural success, having provided the stock for the greater part of the crops of peninsular India. *G. herbaceum*, though it has spread rather widely in cultivation, has had only limited agricultural success. Only in the *wightianums* of western India has it become established in the extensive crops of modern agriculture.

4. DISCUSSION

Hutchinson, Silow and Stephens (1947) pointed out that the basic distinguishing feature of the true cottons is the possession of convoluted lint hairs on the seed. These true lint hairs can be spun, whereas the simple seed hairs of the wild species cannot. They supposed that convoluted hairs arose from simple hairs by mutation in a wild species, and that such a mutant type provided the basis for the evolution under human selection of the true cottons. It is now evident that the convoluted lint hair was established as a characteristic of a truly wild plant without the selective advantage of usefulness to man. The whole of the subsequent development of the cultivated diploid cottons of both species can, however, be accounted for by evolution under human selection.

Rigorous proof of a theory of evolutionary development is rarely possible, but strong support is gained for it if it is used successfully for prediction. In the present instance there are certain consequences of the theory of the recent development of the cottons that can be put to the test. If *G. herbaceum* and *G. arboreum* had been separately domesticated from long established wild plants, the differentiation of the modern cottons would be no more than the most recent episode in the long history of each species. It would then be expected that the genotypic difference between the species would be large compared with that between races of the same species. On the theory that the two species were evolved under domestication from a common stock, however, the genotypic differences between and within species would be of a not very different order, and estimates of the magnitude of the species difference might be very different according to whether primitive or advanced stocks were used in the experiment.

The evidence on which the magnitude of the species distinction has been assessed is worth a brief review. Forms of *G. herbaceum* race *wightianum* are to be found growing mixed with *G. arboreum* race *bengalense* in western India, and with *G. arboreum* race *indicum* in Madras. Similar mixtures of *G. herbaceum* race *kuljianum* and *G. arboreum* race *sinense* have been reported from western China (Yu, quoted by Silow, 1944). In these mixed crops the two species maintain their integrity in spite of some crossing and the appearance of vigorous, fertile F_1 hybrids, and it thus appears that the species distinction is of a different order from the distinctions between races of the same species. The validity of the argument is amply supported by data from experimental material. Genetic breakdown in F_2 and later generations has been demonstrated in crosses between *G. herbaceum* race *wightianum* and *G. arboreum* race *indicum*, and between *G. herbaceum* race *persicum* and *G. arboreum* race *bengalense*. Moreover, attempts to produce agriculturally acceptable cottons from *G. herbaceum* race *wightianum* × *G. arboreum* race *indicum* crosses have failed repeatedly. All this information, however, has been collected from crosses between the most advanced agricultural stocks of the two species. No data are available from crosses between primitive perennials of *G. herbaceum* race *acerifolium* and *G. arboreum* race *indicum*, and if the belief that these represent an early stage in the separation of the species is correct, it is to be expected that such crosses would reveal much less genetic differentiation than has been found in crosses of more advanced stocks.

5. SUMMARY

1. New evidence on the relationships of the wild species of *Gossypium* of Asia and Africa is discussed. It is shown that the theory that the cultivated diploid cottons arose in cultivation in the lower Indus valley from a wild species brought from the eastern Sudan or Arabia is no longer tenable.

2. Possible areas from which the ancestor of the diploid cottons might have come are surveyed, and it is shown that the most likely is the arid bushveld area of southern Africa stretching from Ngamiland to southern Portuguese East Africa. In this region *G. herbaceum* race *africanum* grows wild in natural vegetation.

3. The theory that *G. herbaceum* race *africanum* is an escape from former cultivations is reconsidered in the light of fresh historical evidence, and is shown to be without real support.

4. Recent research on the Red bollworm of cotton is considered, and it is shown that the evolution of *Diparopsis castanea*, which is endemic in southern Africa, can only have taken place on *G. herbaceum* race *africanum*, and it follows that *G. herbaceum* race *africanum* must have been established in southern Africa during the whole evolutionary history of the pest. It must, therefore, be truly wild and not a recent escape from cultivation.

5. The status of *G. arboreum* is considered, and it is shown that there is good reason to believe that it arose in cultivation by differentiation from *G. herbaceum*.

6. The history of the diploid cottons is interpreted in terms of three major assemblies of cultivated forms.

(i) The oldest, and least important in modern agriculture, is the assembly comprising the four cultivated races of *G. herbaceum*, races *acerifolium*, *wightianum*, *persicum* and *kuljianum*.

(ii) In *G. arboreum*, race *indicum* embraces the oldest forms and those most closely related to *G. herbaceum*. It provides the cottons for most of the commercial crop of Peninsular India.

(iii) The most recent, and agriculturally the most important of the three, is what may be called the northern Indian assembly, comprising four races of *G. arboreum*, races *burmanicum*, *soudanense*, *bengalense* and *sinense*. Of these, the two annual races, *bengalense* and *sinense*, provide the cottons that contribute the great bulk of the crops of northern India and China.

6. APPENDIX

Note on the Sections, Species and Races of Gossypium *discussed in the text*

The classification of the species and races of *Gossypium* has been progressively modified in recent years. The basic classification is that given by Hutchinson, Silow and Stephens (indicated below by H.S.S.) in *The Evolution of Gossypium*. Geographical distributions are given there also. Rearrangement below the species level has been proposed in several more recent papers, in which also some adjustments

of distribution have been made in the light of recent additions to knowledge. A list of the Sections, species and races of *Gossypium* discussed is given below, together with references to the papers in which the names used were defined.

Section	Species	Race	Reference
ANOMALA	...	...	H.S.S.
	G. anomalum	...	H.S.S.
	G. triphyllum	...	Douwes and Cuany, 1952
STOCKSIANA	...	...	H.S.S.
	G. stocksii	...	H.S.S.
	G. areysianum	...	Douwes and Cuany, 1952
	G. somalense	...	Knight, 1949 ; Douwes, 1951
HERBACEA	...	...	H.S.S.
	G. herbaceum	*africanum*	Hutchinson, 1949 ; 1950
		acerifolium	Hutchinson, 1949 ; 1950
		wightianum	Hutchinson, 1950
		persicum	Hutchinson, 1950
		kuljianum	Hutchinson, 1950
	G. arboreum	*indicum*	Hutchinson, 1949 ; 1950
		soudanense	Hutchinson, 1949 ; 1950
		burmanicum	H.S.S.
		bengalense	H.S.S.
		sinense	H.S.S.

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SOME DATA ON SEGREGATION IN *SACCHAROMYCES*

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I. INTRODUCTION

SEGREGATION in yeast has been described for many characters since Winge and Laustsen's first demonstration of segregation for morphological properties (1937). In 1949, Lindegren (1949*a*) presented the first chromosome maps of *Saccharomyces*. Frequently, the pairs of characters studied segregated in a 2 : 2 ratio within the yeast ascus, thus indicating simple, monohybrid inheritance (*e.g.* Pomper and Burkholder, 1949).

In a number of instances, however, complex segregations were encountered. Some of these could readily be accounted for on the basis of multiple genes.† Thus, Lindegren and Lindegren (1947) were able to explain complex segregations for the formation of pink pigment as a result of the cooperation of two pairs of genes controlling requirements for adenine and methionine, respectively. Winge and Roberts (1948, 1950*b*, 1952) and Gilliland (1949) showed that simultaneous segregation of several polymeric genes was responsible for the 3 : 1 and 4 : 0 (in addition to 2 : 2) ratios observed for ability to ferment maltose, raffinose and sucrose. Thorne (1951) interpreted in a similar way segregations encountered in a study of the inheritance of flocculent growth.

On the other hand, a number of occasional deviations from the simple 2 : 2 ratio have been reported which are not easily explained in terms of multiple genes. For interpreting unexpected ratios obtained from crosses which usually give 2 : 2 segregations, orthodox mutation has been invoked now and then. An alternative hypothesis has been advanced by Winge and Roberts (1950*a*) who ascribed the observed deviations to a chance survival of four nuclei from an originally eight-nucleate complement during ascospore formation.

For interpreting two tetrads of *Schizosaccharomyces pombe* which segregated aberrantly for the alleles controlling homothallism and

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† The term "multiple gene segregation" will be used in this paper for describing simultaneous segregation of two or more pairs of alleles co-operating in the control of one pair of characters, regardless of the type of interaction (polymeric, complementary, etc.) involved.

heterothallism in this yeast (see Leupold, 1950), an explanation in terms of a triploid or at least trisomic condition of the ascus mother cells was found necessary (Leupold, 1948, unpub.). Each tetrad appeared to segregate into two diploid and two haploid spores. The diploidy of some of the segregants was confirmed (*a*) by analysing their haploid offspring, and (*b*) by crossing one of the diploid segregants to a haploid clone, which led to segregations expected from a triploid. Roman, Hawthorne and Douglas (1951), in reporting on a tetraploid segregation in *Saccharomyces*, have pointed out that from meiosis in polyploids, 4 : 0, 3 : 1, 2 : 2, 1 : 3 and (in the case of triploids, as a result of occasional loss of chromosomes) even 0 : 4 segregations may be expected. Polyploid segregations in *Saccharomyces* have further been described by Lindegren and Lindegren (1951) and Pomper (1952).

Several known genetic mechanisms may thus result in complex segregations in yeast. However, convinced that they are not sufficient to account for many of the observed deviations from the 2 : 2 ratio, Lindegren (1949*a*, 1953) has in addition postulated " gene conversion ", a mechanism which, by the transfer of genic material from one gene to its allele in the heterozygous condition, may endow the recessive gene with capacities of the dominant allele and vice versa. Though capable of explaining any complex segregation, this hypothesis is difficult to verify except by way of exclusion of strictly Mendelian interpretations. Of these, control by multiple genes, as well as chance survival of four nuclei from an originally eight-nucleate complement, have been ruled out as general explanations (Lindegren, 1949*a*; Mundkur, 1950). In Mundkur's (1949) study of aberrant segregations for galactose and melibiose fermentation, the intervention of orthodox mutations as a possible cause of deviations from the expected ratios was minimised by reducing the time elapsing between germination of the ascospores and application of the fermentation test. Mundkur did not exclude, however, polyploidy as a possible mechanism causing the observed deviations from the 2 : 2 ratio, since (as pointed out by Roman and co-workers) not only 3 : 1 and 4 : 0, but also 1 : 3 and even 0 : 4 segregations may be expected from polyploids.

It is the purpose of this paper to present some further data on segregation in *Saccharomyces cerevisiae*. These data were obtained in the course of work on the spontaneous formation of the mutant " petite colonie " (Ephrussi and Hottinguer, 1951), as a by-product of crosses designed to introduce a number of marker genes into our stocks.

2. MATERIAL AND METHODS

(*a*) *Strains and markers*

The following marker pairs were introduced into our stocks : Independence of dependence on : histidine (*HIS/his*), tryptophane (*TRY/try*), methionine (*MET/met*), uracil (*UR/ur*), and adenine (*AD/ad*) ; ability/inability to ferment galactose

(*G*/*g*) ; and disperse/flaky growth in liquid yeast extract—glucose medium (*D*/*d*).* The mating type alleles, *a* and α,† were used as additional markers. These marker pairs were derived from the following strains :

160 (α), *B 15* (*a*) and *868/3b* (α) (*HIS TRY MET UR AD g* ; haploid descendants of " Boulangerie II ", a diploid obtained from the Pasteur Institute in Paris).

175/6b p_1 (*a HIS TRY MET UR ad G* ; haploid descendant of " Yeast Foam " (see Ephrussi, Hottinguer and Tavlitzki, 1949) ;

R 2 (*α his try MET UR AD G* ; a haploid obtained from Dr H. Roman).

P 3 (*aα HIS HIS TRY try MET met UR ur AD AD G g D d* ; a diploid obtained from Dr S. Pomper, originally prepared by crossing : *α HIS TRY met UR AD G d*×*a HIS try MET ur AD g D*).

L-41/2d (*a his try MET UR AD g* ; haploid segregant of (*B 15*×*R 2*)).

L-167/3a (*α his try MET UR AD g* ; haploid segregant of (*868/3b*×*L-41/2d*)).

P 3/5a (*a d*) and *P 3/14c* (*α D*) (*HIS TRY met ur AD G* ; haploid segregants of *P 3*).

(*b*) *Determinations of mating type, growth factor requirements and ability to ferment galactose*

Mating type is determined by mixing two drops of a 24-48 hour liquid culture of the strain to be tested with two drops of a 24-48 hour liquid culture of a test strain of known mating type. The mixture is added to 2 ml. of liquid yeast extract medium (Difco Yeast Extract 0·5 per cent., glucose 3 per cent.) in a test tube and incubated for 24 hours at 25° C.

Requirements for histidine, tryptophane, methionine and uracil are determined by streaking the strains to be tested on solid synthetic media, designated as " histidine free ", " tryptophane free ", " methionine free ", " uracil free ", and " complete ". The complete medium is based on Wickerham's formula (Wickerham, 1946 ; Wickerham and Burton, 1948), but differs from it in that six of its growth factors are replaced by adenine and uracil. The formula for 1000 ml. of " complete " medium is the following :

KH_2PO_4 : 1·0 g. $MgSO_4.7H_2O$: 0·5 g. NaCl : 0·1 g. $CaCl_2.2H_2O$: 0·1 g. $(NH_4)_2SO_4$: 3·5 g. L-Asparagine : 1·5 g. Glucose : 10·0 g.

H_3BO_3 : 500 γ. $CuSO_4.5H_2O$: 40 γ. KI : 100 γ. $FeCl_3.6H_2O$: 200 γ. $MnSO_4.1H_2O$: 400 γ. $Na_2MoO_4.2H_2O$: 200 γ. $ZnSO_4.7H_2O$: 400 γ.

Biotin : 2γ. Calcium pantothenate : 400 γ. Thiamine hydrochloride : 400 γ. L-Histidine monohydrochloride.$1H_2O$: 10 mg. DL-Tryptophane : 20 mg. DL-Methionine : 20 mg. Uracil : 20 mg. Adenine : 20 mg.

Difco Bacto Agar : 20 g.

The media " histidine free ", " tryptophane free ", etc. differ from the " complete " medium in that they lack histidine, tryptophane, etc., respectively.

Cell material for the growth factor tests is obtained from streak cultures on yeast extract agar. One small loopful of cell material is suspended in 10 ml. of Ringer's solution, and one large loopful of the suspension is streaked on a sector of a plate containing the desired medium. Each clone is inoculated on at least two media, namely, on the medium lacking the particular growth factor which the strain is tested for, and on " complete " medium which serves as a control. The plates are incubated at 25° C., and the growth on the two media during the following five days is compared.

The adenine requirement is inferred from pigment formation on yeast extract agar containing 8 per cent. glucose. Under these conditions, all adenineless strains (including " petites " which might be present among the segregants ; Tavlitzki, 1951) form a red pigment.

* See Pomper and Burkholder, 1949.

† The symbols *a*/α used in this paper are those introduced by Lindegren and adopted also by Pomper and by Roman. Mating types *a* and α correspond respectively to mating types + and — of Ephrussi.

Ability to ferment galactose is determined on the basis of gas formation in Durham tubes. Each strain is tested in three media : No. 1 : 0·5 per cent. Difco yeast extract ; No. 2 : 0·5 per cent. yeast extract+2 per cent. glucose ; and No. 3 : 0·5 per cent. yeast extract+2 per cent. galactose. Media No. 1 and 2 serve as controls. A large loopful of cells taken from a streak culture on glucose-yeast extract agar is suspended in 2 ml. of Ringer's solution. From the suspension, three drops are inoculated into a test tube containing 5 ml. of medium, part of which fills a small, inverted test tube. The tubes are incubated at 25° C. Strains which do not fill the inverted tube with gas within one week are classified as non-fermenters (*g*), all other strains as fermenters (*G*).*

The classification for disperse/flaky growth (*D*/*d*) is based on macroscopic growth habit in liquid yeast extract-glucose medium (Difco yeast extract 0·5 per cent., glucose 3 per cent. ; cultures incubated at 25° C.).

(c) *Technique of crossing*

Crosses are set up as mass combinations of cells of the two strains to be crossed, in the way described for the mating type tests. One day after the cultures have been combined, a loopful of the mixture is transferred to fresh yeast extract medium, and this is repeated on the second, third and fourth day ; each time, the inoculum is taken from the latest culture. After five days, the culture contains mainly diploid cells. A cell of typical diploid appearance is then isolated with a micromanipulator, and the clone to which it gives rise is transferred to yeast extract agar and therefrom to sporulation medium. Sporulation is induced on Gorodkowa agar or on Adams agar (Adams, 1949). In either case, care is taken to reduce presporulation growth to a few days in order to avoid intervening sporulations as much as possible.

3. RESULTS

(a) *Segregation data*

The data presented below describe segregations in six hybrids heterozygous for various markers, as follows :

1. *C L-41* (B 15 × R 2) (*aα HIS his TRY try G g*) (12 asci)
2. *C L-167* (868/3b × L-41/2d) (*a α HIS his TRY try*) (3 asci)
3. *C 969* (L-167/3a × 175/6b p_1) (*a α HIS his TRY try AD ad G g*) (31 asci)
4. *P 3* (*a α TRY try MET met UR ur G g D d*) (26 asci)
5. *C 948* (P 3/5a × 160) (*a α MET met UR ur G g D d*) (29 asci)
6. *C 967* (P 3/14c × 175/6b p_1) (*a α MET met UR ur AD ad*) (33 asci)

The numbers of analysed four-spored asci in which all four spores germinated are indicated in parentheses for each hybrid.

The frequencies of various segregation types observed in each hybrid are indicated in table 1. It is seen that a majority of 2 : 2 segregations was obtained for mating type (132 out of 134 asci) ; for ability to synthesise histidine (44/46), tryptophane (65/72), uracil (87/88) and adenine (62/64) ; and for growth habit (disperse/flaky) (53/55). 2 : 2 segregations were somewhat less frequent for the ability to grow in the absence of methionine (75/88) and for the

* The authors wish to thank Dr B. Ephrussi for performing all fermentation tests.

TABLE 1

The phenotypic segregations (for details see text)

Hybrid no.	*a* : α	*HIS* : *his*	*TRY* : *try*	*MET* : *met*	*UR* : *ur*	*AD* : *ad*‡	*G* : *g*	*D* : *d*	Total
C L-41	12 (2 : 2)	12 (2 : 2)	1 (3 : 1) 10 (2 : 2) 1 (1 : 3)	...	...	...	9 (1 : 3)§ 1 (0 : 4)§	...	12
C L-167	3 (2 : 2)	3 (2 : 2)	3 (2 : 2)	...	...	...	...	...	3
C 969	30 (2 : 2) 1 (1 : 1 : 2*nm*)*	2 (3 : 1) 29 (2 : 2)	1 (3 : 1) 30 (2 : 2)	...	...	1 (4 : 0) 30 (2 : 2)	1 (4 : 0) 1 (3 : 1) 29 (2 : 2)	...	31
P 3	26 (2 : 2)	...	4 (3 : 1) 22 (2 : 2)	26 (2 : 2)	26 (2 : 2)	...	26 (2 : 2)	26 (2 : 2)	26
C 948	29 (2 : 2)	...	...	18 (2 : 2)† 11 (1 : 3)†	29 (2 : 2)†	...	Clear-cut classification impossible ‖	2 (3 : 1) 27 (2 : 2)	29
C 967	32 (2 : 2) 1 (0 : 0 : 4*nm*)*	...	...	1 (4 : 0) 31 (2 : 2) 1 (1 : 3)	1 (3 : 1) 32 (2 : 2)	1 (3 : 1)‡ 32 (2 : 2)‡	...	...	33
Total	*132* (2 : 2) *1* (1 : 1 : 2*nm*)* *1* (0 : 0 : 4*nm*)*	*2* (3 : 1) *44* (2 : 2)	*6* (3 : 1) *65* (2 : 2) *1* (1 : 3)	*1* (4 : 0) *75* (2 : 2) *12* (1 : 3)	*1* (3 : 1) *87* (2 : 2)	*1* (4 : 0) *1* (3 : 1) *62* (2 : 2)	*1* (4 : 0) *1* (3 : 1) *55* (2 : 2) *9* (1 : 3) *1* (0 : 4)	*2* (3 : 1) *53* (2 : 2)	134

* *nm* = No mating reactions, neither with strains of mating type *a* nor with strains of mating type α.

† Ratios indicating growth reactions on methionine free and uracil free medium only. On complete medium, 11 (4 : 0) and 18 (3 : 1) segregations were obtained in *C 948*.

‡ Determined on the basis of pigment formation on yeast extract agar containing 8 per cent. glucose. On synthetic complete medium, *C 967* yielded the following segregations : 7 (4 white : 0 red), 20 (3 white : 1 red), 6 (2 white : 2 red).

§ Because of loss of two segregants of *C L-41*, complete fermentation data could be obtained for 10 out of 12 asci only.

‖ In *C 948*, a clear-cut classification into galactose fermenters and galactose non-fermenters was not found possible. Four days after inoculation, only two clones per ascus had started fermentation, but one week after inoculation, gas formation was observed in the majority of the strains. No detailed study of the effect was undertaken.

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ability to ferment galactose (55/67, not counting 29 asci of *C 948* in which the 2 : 2 ratio was frequently obscured by the appearance of slow fermenters).

The deviations from the 2 : 2 ratio which have been observed (see table 1) may be classified as follows :

(i) *Systematic deviations, occurring in an important fraction of the asci obtained from one hybrid and concerning one particular pair of characters only.* This group includes the segregations for ability to ferment galactose in hybrid *C L-41* (no 2 : 2 segregation among 10 asci), for pigment formation on synthetic medium in hybrid *C 967* (only 6 2 : 2 segregations among 33 asci), and for growth on methionine free and complete medium in hybrid *C 948* (on methionine free medium, only 18 2 : 2 segregations among 29 asci). In each of these cases, the observed deviations are probably due to simultaneous segregation of multiple genes controlling the pair of characters concerned. For a more detailed discussion, the reader is referred to paragraph 3*c*.

(ii) *Systematic deviations, occurring in a few asci only but concerning most or all pairs of characters involved.* Two clear-cut cases of this kind have been observed, namely, ascus *27* of hybrid *C 967* (deviations for all characters involved : 4 non-maters, 3 *AD* : 1 *ad*, 4 *MET* : 0 *met*, 3 *UR* : 1 *ur*), and ascus *16* of hybrid *C 969* (deviations for four out of the five characters involved : 2 *a* : 2 *α*, 4 *AD* : 0 *ad*, 3 *HIS* : 1 *his*, 3 *TRY* : 1 *try*, 4 *G* : 0 *g*). In both cases, the observed segregations could be shown to be due to polyploidy of the ascus mother cell concerned (Leupold, in press ; for main results, see paragraph 3*d*). The same interpretation is suggested in the case of a third ascus, *C 969/5*, by the presence of two non-maters (in addition to one *a* and one *α* clone) among the four single spore strains, combined with a 3 : 1 segregation for histidine dependence. This ascus was not studied in detail.

(iii) *Occasional deviations, occurring in a few asci and concerning one pair of characters per ascus only* (in several cases, however, coinciding with systematic deviations of type (i) concerning a second pair of characters). Occasional deviations from the 2 : 2 ratio have been encountered for several marker pairs, as follows :

Hybrid *C L-41* : 1 (3 *TRY* : 1 *try*) and 1 (1 *TRY* : 3 *try*).
Hybrid *C 969* : 1 (3 *G* : 1 *g*). Hybrid P *3* : 4 (3 *TRY* : 1 *try*).
Hybrid *C 948* : 2 (3 *D* : 1 *d*). Hybrid *C 967* : 1 (1 *MET* : 3 *met*).

No detailed study of these asci has been undertaken. Among several interpretations which might account for the observed segregations, the possibility of mutation should especially be taken into consideration.

(*b*) *Linkage relations*

On the basis of the segregations described above, an attempt was made to determine the linkage relations between the genes involved. Only asci segregating 2 : 2 for all characters concerned were taken

as the basis for the calculations, with the following exceptions : (i) The asci obtained from the hybrids *C L-41* and *C 948* were excluded from the calculations only as far as linkage between the ability to ferment galactose and the other characters was concerned. (ii) In the case of the 11 asci of *C 948* which showed deviating segregations for the ability to grow on methionine free medium, the calculations were based on the interpretations given in paragraph 3*c*.

The cross $AB \times ab$ leads to three different types of asci : Type I (ditype) : 2 *AB* : 2 *ab*. Type II (ditype) : 2 *AB* : 2 *ab*. Type III (tetratype) : 1 *AB* : 1 *Ab* : 1 *aB* : 1 *ab*. Independent segregation of the two loci is indicated by equal frequencies of type I and II, linkage by type II being considerably less frequent than type I. If the two loci segregate independently of each other and one or both loci segregate independently of their centromeres, a ratio of I : II : III = 1 : 1 : 4 is expected. Relative frequencies of type III asci lower than 2/3, combined with equal frequencies of types I and II, suggest independence between the two loci but close linkage between each locus and its centromere.

In unordered tetrads (like those of *Saccharomyces*), in which the products of meiosis are not linearly arranged, the distance from the centromere cannot be determined directly for one particular locus. It is possible, however, to calculate the proportions of second-division segregation for three independent loci if the relative frequencies of tetratype asci are known from each of the three possible crosses, $AB \times ab$ (or $Ab \times aB$), $AC \times ac$ (or $Ac \times aC$), and $BC \times bc$ (or $Bc \times bC$) (Perkins, 1949, and Whitehouse, 1950 ; a graphical method was developed by Lindegren, 1949*a*). The frequencies of second-division segregations so obtained may finally be converted into ordinary recombination frequencies (Spiegelman, 1952 ; Papazian, 1952).

Table 2 gives the frequencies of the three tetrad types for each pair of loci studied. Data from different crosses involving each pair of characters, whether in coupling or repulsion, are lumped together. On the other hand, data concerning tryptophane dependence have been separated according to the origin of the strains introducing tryptophane deficiency. This character has been derived partly from material obtained from Dr H. Roman (strain *R 2*) and partly from material supplied by Dr S. Pomper (strain *P 3*). Since the corresponding genes are not necessarily identical, they are designated with the symbols TRY_R/try_R and TRY_P/try_P, respectively. The same holds for the segregation data involving inability to ferment galactose, a character which was partly derived from strains isolated in this laboratory (strain *160*, *B 15* and *868/3b* ; descendants of strain " Boulangerie II ") and partly from strain *P 3* which was received from Dr Pomper. The corresponding genes, which again need not be identical, are referred to by the symbols G_E/g_E and G_P/g_P respectively.

In the third column of table 2, χ^2 and P are given on the assumption that the observed numbers of type I and II tetrads represent random

samples of a population in which the two types are equally frequent. It can be seen that close linkage is indicated only for *HIS/his* and TRY_R/try_R ($P<0{\cdot}001$, recombination value = 19·5 per cent.). It should be mentioned, however, that thus far only part of the possible gene combinations have been realised.

In the fourth column of table 2, χ^2 and P are calculated on the assumption that the observed frequencies of the three tetrad types were obtained as random samples from a population in which the

TABLE 2

Linkage relations. (*For explanation, see text*)

Cross	Frequency of tetrad types			χ^2 and P for I : II = sample from basic ratio 1 : 1		χ^2 and P for I : II : III = sample from basic ratio 1 : 1 : 4	
	I	II	III	χ^2	P	χ^2	P
$a\times HIS$	9	10	22	0·05	0·8	3·20	0·2
$a\times TRY_R$	5	11	25	2·25	0·1	3·23	0·2
$a\times TRY_P$	6	5	11	0·09	0·8	2·86	0·2
$a\times MET$	12	17	53	0·86	0·4	1·09	0·6
$a\times UR$	15	17	50	0·13	0·7	1·35	0·5
$a\times AD$	11	11	37	0	1·0	0·39	0·8
$a\times G_E$	7	2	19	2·78	0·1	2·70	0·3
$a\times G_P$	1	6	15	3·57	0·05	3·43	0·2
$a\times D$	7	7	35	0	1·0	0·51	0·8
$HIS\times TRY_R$	25	0	16	25·0	<0·001	...	...
$HIS\times AD$	4	5	19	0·11	0·7	0·41	0·8
$HIS\times G_E$	3	6	19	1·00	0·3	0·99	0·6
$TRY_R\times AD$	4	5	19	0·11	0·7	0·41	0·8
$TRY_R\times G_E$	3	7	18	1·60	0·2	1·76	0·4
$TRY_P\times MET$	4	4	14	0	1·0	0·09	0·95
$TRY_P\times UR$	1	4	17	1·80	0·2	2·34	0·3
$TRY_P\times G_P$	2	6	14	2·00	0·2	2·27	0·3
$TRY_P\times D$	1	4	17	1·80	0·2	2·34	0·3
$MET\times UR$	14	13	55	0·04	0·9	0·04	0·98
$MET\times AD$	6	7	18	0·08	0·8	0·60	0·7
$MET\times G_P$	2	4	16	0·67	0·4	0·91	0·6
$MET\times D$	10	10	29	0	1·0	1·23	0·5
$UR\times AD$	6	7	18	0·08	0·8	0·60	0·7
$UR\times G_P$	4	2	16	0·67	0·4	0·91	0·6
$UR\times D$	11	9	29	0·20	0·7	1·47	0·5
$AD\times G_E$	3	3	22	0	1·0	1·79	0·4
$G_P\times D$	4	3	15	0·14	0·7	0·16	0·9

ratio I : II : III was 1 : 1 : 4. It is obvious that in no case the observed deviations from the basic ratio are sufficiently important to suggest close linkage between a locus and the centromere. Precise calculations following the method of Whitehouse would therefore be of little value. The absence of ratios deviating significantly from the 1 : 1 : 4 ratio does not exclude, however, close linkage of at least some loci with their respective centromeres, for two reasons: (i) larger samples might have revealed significant deviations which remained

undetected with the small samples actually studied ; (ii) tetratype frequencies smaller than 2/3 are obtained only in cases where *both* genes are closely linked to their centromere ; it is not excluded, therefore, that at least one of the genes (or any two independent genes the simultaneous segregations of which have not yet been studied) were closely associated with the centromere.

It is not possible, from these data, to decide whether the two genes for tryptophane deficiency, try_R and try_P, are identical. The same applies to the two genes for inability to ferment galactose, g_E and g_P.

Lindegren's segregation data involving mating type, methionine dependence, adenine dependence and galactose fermentation (Lindegren, 1949*a*) agree with our results concerning the same characters in as far as they suggest absence of linkage between the loci involved. However, pairwise comparison of the frequencies of tetratype asci obtained in the crosses $a \times MET$, $a \times AD$, $a \times G_E$, $a \times G_P$, $MET \times AD$, $MET \times G_P$ and $AD \times G_E$, with the corresponding tetratype frequencies reported by Lindegren for his gene pairs a/α, MT/mt, AD/ad and G/g, reveals a significant difference in the combination $AD \times G_E$ (Lindegren's data : 45/147 in the cross $AD \times G$; our data : 22/28 in the cross $AD \times G_E$; $P<0{\cdot}001$). It cannot be decided, on the basis of these comparisons, whether our genes *a*, *met* and g_P are identical with the corresponding genes *a*, *mt* and *g* described by Lindegren. It is probable, however, that of the two genes g_E and *ad*, at least one is different from the corresponding genes *g* and *ad* present in Lindegren's material. Our results are in agreement with the assumption of independent segregation between at least one of the two loci and its centromere, whereas Lindegren's data indicate close linkage between each of the two loci and their centromeres (approximate distances from centromere : 6 units for *g* and 11 units for *ad*, determined by including tetratype frequencies which involve a/α as a third locus).

Such differences are not surprising, since the genes *g* and *ad* described by Lindegren and the corresponding genes used in our work have different origins. In the case of galactose fermentation it is even probable, from the results described in the following paragraph, that more than one gene is involved in the control of this character.

(c) *Multiple gene segregations*

Galactose.—Out of ten asci obtained from hybrid *C L-41*, nine contained one galactose fermenter and three non-fermenters, and one ascus yielded four non-fermenters. This suggests that two complementary genes controlling the fermentation of galactose are involved in cross ($B\ 15 \times R\ 2$), and that ability to ferment this sugar requires the simultaneous presence of the two dominant genes. Since

R 2 is a galactose fermenter, the two recessive genes have been introduced into the cross presumably by *B 15*.

These results represent the first case suggesting a complementary instead of a polymeric control of sugar fermentation. A biochemical basis for a complementary mechanism of control may be found in the results of Leloir and his colleagues (Trucco and co-workers, 1948 ; Caputto and co-workers, 1949, 1950 ; Cardini and co-workers, 1949), who showed that at least three enzymatic steps are involved in the conversion of galactose into an intermediate which is common to the glucozymase complex. Each step is catalysed by an enzyme which in turn may be assumed to be controlled by a gene. If the

TABLE 3

Segregation in hybrid C 948 *: The three types of segregation for ability to grow on methionine free, uracil free and complete medium, represented by three typical asci*

Strain no.	Growth on :			Genotype
	Methionine free	Uracil free	Complete	
14a	+	−	+	*MET ur C*
b	−	+	+	*met UR c*
c	−	−	+	*met ur C*
d	+	+	+	*MET UR c*
13a	+	+	+	*MET UR*
b	−	+	+	*met UR*
c	−	−	−	*met ur c*
d	+	−	+	*MET ur C*
15a	−	+	+	*met UR*
b	−	−	−	*MET ur c*
c	+	+	+	*MET UR*
d	−	−	+	*met ur C*

formation of each of the three enzymes required the presence of the dominant at one of the corresponding three loci, then a hybrid heterozygous for two or three genes would be expected to yield 2 : 2, 1 : 3 and 0 : 4 segregations. Of these ratios, the latter two have actually been encountered in *C L-41*, and the absence of tetrads yielding a 2 : 2 ratio may be due to the small number of asci studied.

Adenine, methionine and pigment formation.—In hybrid *C 967*, regular segregation for the ability to synthesise adenine was shown by 2 : 2 segregations for the formation of red pigment. This, however, was true only for the cultures which were grown on yeast extract agar containing 8 per cent. glucose ; one exceptional 3 : 1 segregation (ascus *C 967/27*) will be discussed in paragraph 3*d*. On "complete" synthetic medium, pigment formation was found to depend on

methionine as well as on adenine dependence, for the recombinants carrying both recessives remained white. This led to 4 : 0 segregations (7 ditype asci containing the two recombinant genotypes : *AD MET* and *ad met*) and 3 : 1 segregations (18 * tetratype asci containing 1 *AD MET* : 1 *AD met* : 1 *ad met* : 1 *ad MET*) in addition to 2 : 2 segregations for white and red (6 ditype asci yielding the two parental genotypes : *AD met* and *ad MET*). This dependence of pigment formation by adenineless clones on the ability to synthesise methionine has been reported earlier by Lindegren and Lindegren (1947).

Uracil.—A co-operation of two pairs of genes is also probable in the complex segregations observed in hybrid *C 948*. Comparison of the results of growth factor tests carried out on methionine free, uracil free and " complete " medium revealed the existence of three types of asci. Table 3 gives the results for one typical ascus of each group. In 11 out of 29 asci, 2 : 2 segregation for the ability to grow on methionine free medium was accompanied by the ability of all four segregants to grow on complete medium. In a second group of 7 asci, 2 : 2 segregation was observed on methionine free medium but one of the two methionineless segregants of each ascus failed to grow on complete medium. A last group of 11 asci segregated 1 : 3 on methionine free and 3 : 1 on complete medium ; it was always one of the three methionine-negative segregants which failed to grow on complete medium. The 18 aberrant clones which were unable to utilise the two media were also unable to grow on uracil free medium. Since, on the latter, 2 : 2 segregation was observed in all 29 asci, it was concluded that all aberrant segregants carried the gene for uracil deficiency.

A possible interpretation of these findings is as follows. It may be assumed that the gene " *ur* " present in the strain *P 3/5a* (one of the parents of hybrid *C 948*) blocks the synthesis of an unknown growth factor X rather than of uracil. However, due to the presence of a gene *C*, *P 3/5a* is able to utilise exogeneous uracil which it converts into X. Strain *160* (the other parent of hybrid *C 948*) is unable to carry out this conversion because it contains the allele *c*. On the other hand, it is independent of uracil since the presence of the gene " *UR* " permits direct synthesis of the growth factor X. The two loci are linked. In tetrads which have not undergone crossing-over between the two loci, the two parental genotypes *UR c* and *ur C* will segregate in a 2 : 2 ratio. Phenotypically, this results in two segregants being able to grow both on uracil free and on complete medium, and in two segregants which grow on the latter but not on the former medium. In tetrads in which one crossing-over has taken

* Excluded are two asci in which the 3 : 1 segregation for pigment formation was combined with deviations in the segregation of *AD/ad* and *MET/met*, namely, with a 1 : 3 segregation for *MET/met* in ascus *C 967/23*, and with 3 : 1 and 4 : 0 segregations for *AD/ad* and *MET/met*, respectively, in ascus *C 967/27* (see paragraph 3*d*). In both cases, pigment formation on synthetic medium was confined to strains combining dependence on adenine with independence of methionine.

place between the two loci, two recombinant genotypes are obtained in addition to the two parental ones, as follows :

	UR C	*UR c*	*ur C*	*ur c*
Growth on uracil free medium .	+	+	o	o
Growth on complete medium .	+	+	+	o

Of the two recombinant types, one is able to manufacture X directly as well as to convert uracil into X, whereas the other one has lost both activities. It is this last type of strains which does not grow even on " complete " medium since the hypothetical growth factor X is not available in any of the synthetic media used. This factor must be present in yeast extract, however, since growth on medium containing yeast extract is normal.

Four strand double crossing-over between the two loci in question would correspondingly result in a 2 : 2 segregation for the two recombinant genotypes. Phenotypically, this would lead to 2 : 2 segregations both on uracil free and on complete medium. The rarity of double crossing-overs is probably responsible for the fact that this last type of segregation has not been observed in our material.

Segregation on methionine free medium is disturbed only in tetrads in which recombination has taken place and in which the recombinant genotype *ur c* happens to be combined with *MET*. If we assume that in every one of the 11 asci in which 1 : 3 segregation has been obtained on methionine free medium, the one segregant which does not grow on any medium is methionine positive, segregation for *MET/met* is perfectly regular.

In the last column of table 3, the interpretation given to the phenotypical segregations is indicated for three typical asci. As to the alleles *C/c*, complete interpretation is only possible for asci in which the parental genotypes *UR c* and *ur C* have segregated in a 2 : 2 ratio. In all other asci, *C* and *c* can be distinguished in the uracil negative but not in the uracil positive segregants ; it is to be predicted, however, that in these tetrads, one of the uracil positive clones carries *C*, the other *c*.

(*d*) *Polyploid segregations*

From hybrid *C 967*, an ascus was isolated in which the four characters involved segregated as follows :

Strain no.	Mating type	Synthesis of *Ad*	*Met*	*Ur*
27 a :	no mating reactions	+	+	−
27 b :	no mating reactions	+	+	+
27 c :	no mating reactions	−	+	+
27 d :	no mating reactions	+	+	+

On the basis of results obtained in *Schizosaccharomyces pombe* (Leupold, 1948, unpublished) and of the findings of Roman and co-workers (1951) and of Lindegren and Lindegren (1951) in *Saccharomyces*

cerevisiae, these segregations can be interpreted as resulting from meiosis in a polyploid. Indeed, the four clones isolated from the ascus not only formed cells of size and shape characteristic of diploids, but were also able to sporulate. Analysis of the progeny confirmed the diploidy of the four strains. Twelve asci, three from each of the four clones, were dissected, and the segregations for the four characters involved were studied. The 4 : 0, 2 : 2 and 0 : 4 ratios which were obtained indicated that the spores of ascus *27* had the following constitution :

27 a :	2n :	*a* α *AD ad MET met ur ur*
27 b :	2n :	*a* α *AD ad MET met UR UR*
27 c :	2n :	*a* α *ad ad MET met UR ur*
27 d :	2n :	*a* α *AD AD MET met UR ur*

These genotypes agree with the observations on the phenotypes of the four segregants. They suggest that ascus *27* had originated from a tetraploid cell of the formula :

4n : *a a* α α *AD AD ad ad MET MET met met UR UR ur ur*

The finding of a majority of regular asci in *C 967* suggests that this hybrid (which had been grown from a single cell) was originally a diploid and that it became heterogeneous for ploidy subsequently. The formula suggested for the tetraploid mother cell of ascus *27* represents the double of the diploid genotype of hybrid *C 967*. Of the two mechanisms which could be responsible for such a doubling, namely, copulation of diploid cells and endomitosis, the latter would seem to be more probable since copulation figures have not been observed in diploid clones heterozygous for the mating type alleles.

A second ascus which gave unexpected segregations for most of the markers involved was obtained from hybrid *C 969* :

Strain no.	Mating type	Synthesis of *Ad*	*His*	*Try*	Ferm. of *G*
16 a :	*a*	+	+	+	+
16 b :	*a*	+	−	−	+
16 c :	α	+	+	+	+
16 d :	α	+	+	+	+

Again, the observed ratios suggested segregation in a polyploid, and size and shape of the vegetative cells indicated that the four segregants were diploid. Since none of the clones could be induced to sporulate, segregants *a* and *b* (which in mass combinations with haploid testers behaved as *a* strains) were crossed to segregants *c* and *d* (which in combinations with haploid testers reacted as α strains). Abundant zygote formation was observed in all four crosses, $a \times c$, $a \times d$, $b \times c$, and $b \times d$. The four hybrids obtained from isolated zygotes resembled their parents in the shape of the cells but differed from normal diploids in that the cells were somewhat larger. All four hybrids sporulated abundantly, forming ascospores which were slightly larger than normal. The four hybrids were presumed to be tetraploids and

expected to segregate into diploids. Segregations for the markers involved were studied in a series of asci isolated from the four hybrids. They were in agreement with the segregations expected from tetraploids if the diploid parents had been of the following constitutions :

16 a :	2n :	*a a*	*AD ad*	*HIS his*	*TRY TRY*	*G g*
16 b :	2n :	*a a*	*AD ad*	*his his*	*try try*	*G g*
16 c :	2n :	*α α*	*AD ad*	*HIS his*	*TRY try*	*G g*
16 d :	2n :	*α α*	*AD ad*	*HIS HIS*	*TRY try*	*G g*

These genotypes are in agreement with the phenotypical segregations observed in ascus *16*. They indicate that the ascus originated from a tetraploid cell of genotype :

4n : *a a α α AD AD ad ad HIS HIS his his TRY TRY try try G G g g*,

a constitution which represents the double of the diploid formula of hybrid *C 969*. For the origin of the tetraploid mother cell, the same arguments apply as in the case of ascus *C 967/27* discussed above.

The detailed results of analysis of the aberrant asci *C 967/27* and *C 969/16* will be published separately.

4. SUMMARY

1. Data on segregations of a number of characters (mating type, growth factor requirements, galactose fermentation, and growth habit) in *Saccharomyces cerevisiae* are presented.

2. On the basis of the 2 : 2 segregations obtained, linkage relations are calculated.

3. Complex segregations for galactose fermentation, pigment formation in adenineless strains, and uracil requirement, are interpreted in terms of dihybrid inheritance.

4. Two cases of complex segregations are shown to be due to tetraploidy.

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ANALYSIS OF SUSPECTED HYBRID SWARMS IN THE GENUS *EUCALYPTUS*

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1. INTRODUCTION

For arborescent species with long periods between successive generations and flowers borne on slender branches at great height, it is desirable to develop indirect methods for the study of cross breeding. Such a problem arises frequently in the Australian genus *Eucalyptus*, for which hybrid swarms have often been reported (Brett, 1938; Pryor, 1950). The following method of analysing such swarms differs from most of those previously described (Anderson, 1949) in that it requires a knowledge of the variability of the progeny of individual members of the hybrid swarm rather than a knowledge of the variability of the swarm itself.

2. EXPERIMENTAL MATERIAL

The two tree species, *E. elaeophora* F. Muell. and *E. goniocalyx* F. Muell. have been regarded as closely related by some (Bentham, 1866) and by others as belonging to different sections of the genus (Baker and Smith, 1920; Blakeley, 1934). They are not difficult to distinguish, but at several localities in Victoria they are linked by a series of intermediate forms. The relationship between the two species has been investigated by raising seedlings from selected parent trees and measuring their juvenile characteristics, also by study of the field distribution of the species and their suspected hybrids.

The species names are here accepted and used in the sense in which they are defined in the *Flora of Victoria* (Ewart, 1930), although a study of the type material of each would suggest that the name *E. elaeophora* should be suppressed in favour of *E. goniocalyx* and that the plant generally known as *E. goniocalyx* ought to be redescribed under another name. However, in Australia there exists no confusion as to which species is meant when the current names are used and the author (like Maiden, 1914) does not propose that they should be changed.

(a) Description of species

Eucalyptus goniocalyx is a large tree which may attain a height of up to 200 feet with a diameter of 6-8 feet at breast height. The bark

is smooth, except at the base of the trunk where it may be rough and flaky for several feet. Although usually grey it is sometimes strongly mottled with yellow. Its typical habitat is a well-drained soil, usually on hillsides in areas receiving an annual rainfall of 40-50 inches. Such conditions are common on the seaward slopes of the Eastern Highlands and here *E. goniocalyx* is often a co-dominant of the forest.

Eucalyptus elaeophora, on the other hand, is a small tree seldom growing to more than 80 feet, with a diameter of about 2 feet. It has a rough flaky bark on all but the smallest branches. The usual habitat is on well-drained hillsides in places receiving an annual rainfall of about 18-30 inches. Except for a few localities in South

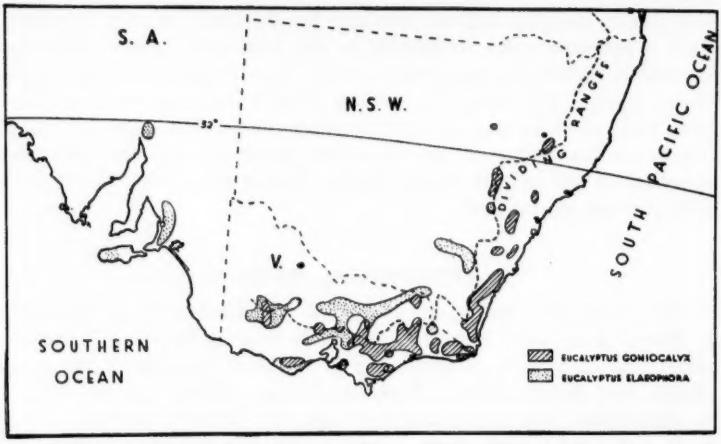

FIG. 1.—The distribution of *Eucalyptus elaeophora* and *Eucalyptus goniocalyx*. (Data in part from Carter, 1945.)

Australia and Southern Victoria, the species is confined to the inland slopes of the Eastern Highlands, fig. 1.

The species differ in several other respects, the most important of which are illustrated in fig. 2.

(*b*) *Field studies*

In the course of mapping the distribution of the Eucalyptus species in the region of the Dandenong Range, Victoria, it was observed that *E. elaeophora* and *E. goniocalyx* graded into each other. So complete was this gradation that it was impossible to define accurately the boundary between the species (Clifford, 1953). Investigation of other areas showed that such intergrading was common where the habitats of the two species adjoined (fig. 3). Intermediate trees have been observed on the Dandenong Range, in several places on the hills about the Kinglake Plateau, and further east on the Cathedral

Range. They are also reported to occur in the Grampian Ranges (L. D. Pryor in litt.).

In zones of intergradation the intermediate trees were variable

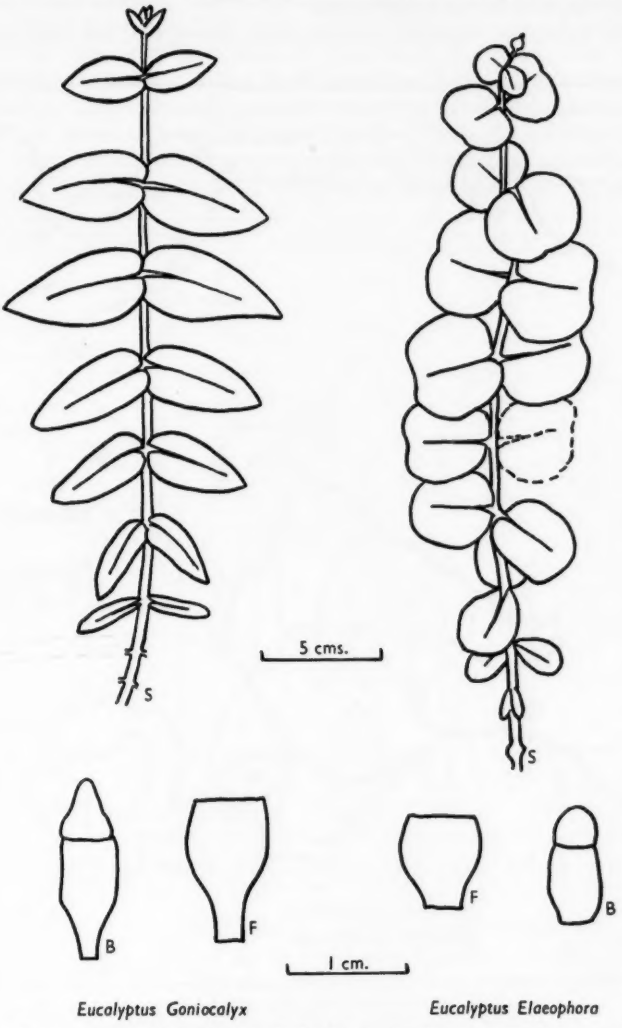

FIG. 2.—Some characteristic features of *Eucalyptus elaeophora* and *Eucalyptus goniocalyx*. S. seedling ; B. bud ; F. fruit.

inter se, some had a habit and bark suggesting an affinity with *E. elaeophora*, others had an appearance suggesting relationship with *E. goniocalyx*. The leaves on seedlings and on coppice shoots arising

from the stools of felled trees in these zones were also very variable in shape. In stands of either *E. elaeophora* or *E. goniocalyx* there was little variability of the coppice foliage between different trees, or in the shape of the seedling leaves.

No transplant experiments have been attempted, but each species

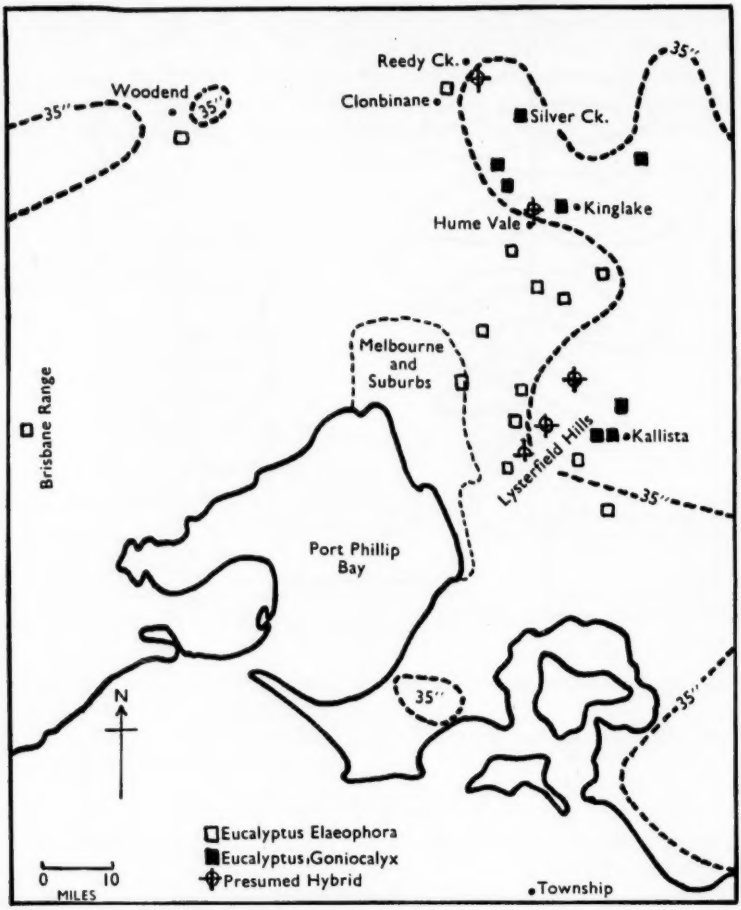

FIG. 3.—The distribution of *Eucalyptus elaeophora*, *Eucalyptus goniocalyx* and forms intermediate between them, in some localities near Melbourne.

has been observed growing in a variety of habitats. In each instance the species has retained its identity, even when grown in the habitat of the other, which confirms that they are not merely environmental forms of the one genotype.

(c) *Progeny studies*

The seedling leaves of *E. elaeophora* and *E. goniocalyx* are very different in shape, and a comparison of these and the seedling leaves of the "intermediate" trees was undertaken. Seeds were collected from several trees typical of either one or the other species and also from trees that were intermediate between them. The seeds were sown in 8 in. pots filled with a friable red-loam and placed in a heated glasshouse. Approximately 30 seeds were sown in each pot and within a fortnight most of these had germinated. When the seedlings had grown about 2 pairs of leaves the number of plants per pot was reduced to twelve. The thinning was designed to leave these twelve plants

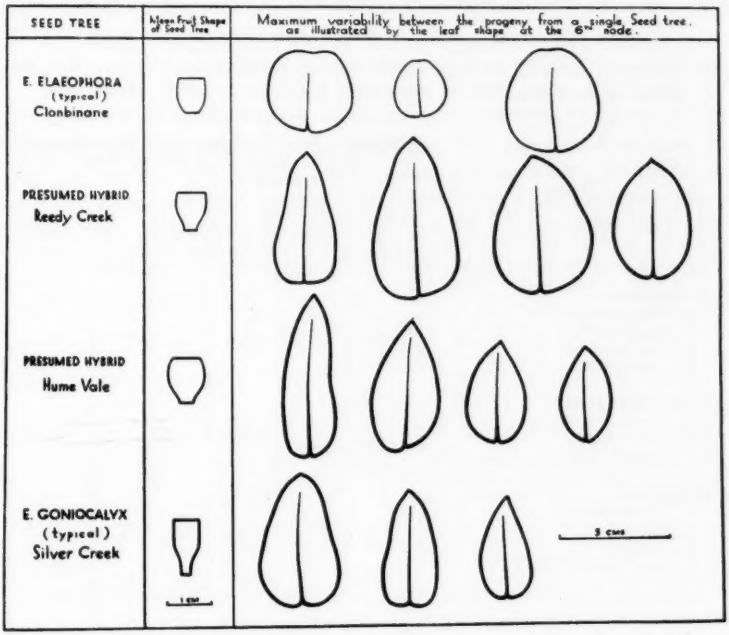
SEED TREE
Mean Fruit Shape of Seed Tree
Maximum variability between the progeny from a single Seed tree, as illustrated by the leaf shape at the 6th node.
E. ELAEOPHORA (typical) Clonbinane
PRESUMED HYBRID Reedy Creek
PRESUMED HYBRID Hume Vale
E. GONIOCALYX (typical) Silver Creek
1 cm.
5 cms.

FIG. 4.—The variability between seedlings from the same parent, as illustrated by the leaf shape at the sixth node.

evenly spaced. No other conscious selection was practised except to remove any very stunted or diseased plants. The seedlings were finally harvested when they showed 6-8 pairs of fully expanded leaves. Altogether the progeny of fourteen trees were raised.

An examination of the seedlings grown indicated that there was less variability between those raised from parents typical of either *E. elaeophora* or *E. goniocalyx*, than between those from parents with an appearance intermediate between those species. This is illustrated in fig. 4, where leaves from the 6th node of four different seedlings

from each of several parents, have been drawn. The leaves were chosen to show the maximum variability between the progeny raised from each parent.

If the seedlings from parents typical of each of the three groups studied were pooled and the variance of the length-breadth ratio of the leaves calculated, it was greater for the progeny of " intermediate " trees than for those from either *E. elaeophora* or *E. goniocalyx* at 5 of the 6 nodes measured. This is shown in table 1, from which it is evident that at the later nodes the variability of the progeny of the intermediate trees is maintained whereas the progeny of each of *E. elaeophora* and *E. goniocalyx* tend to become more uniform.

TABLE 1

The relative variability of the length-breadth ratio of seedling leaves of progeny from trees typical of E. elaeophora, E. goniocalyx *and trees intermediate between them*

Parent	Weighted mean variance of the length-breadth ratio of leaves at successive nodes above the cotyledons					
	1	2	3	4	5	6
E. elaeophora						
1. Yan Yean . . .	0·81	0·29	0·19	0·21	0·14	0·14
2. Clonbinane . . .						
3. Brisbane Rge. . . .						
4. Wattle Glen . . .						
Intermediate trees						
1. Reedy Creek . . .	0·95	0·44	0·31	0·46	0·63	0·49
2. Hume Vale . . .						
3. Lysterfield Hills 1, 2, 3, 4 .						
E. goniocalyx						
1. Silver Creek . . .	0·83	0·44	0·27	0·31	0·28	0·26
2. Kinglake						
3. Kallista						
4. Mt. Wilson, N.S.W. . .						

The analysis of the variability in a population containing species and presumed hybrids may be carried further as follows. Consider the inheritance in pure lines and hybrids, of a character controlled by 4 genes. Assume that the parents (P_1 and P_2) are each homozygous for different alleles ; that the viability and number of gametes produced by each is similar ; that the F_1 hybrid between them is fully fertile ; that the influence of the environment is negligible and that the overall mode of action of the genes is additive on a suitable scale. The mean measurements and their variances for the character in the parents and hybrid derivatives are shown in table 2.

When plotted graphically these values outline a triangle (fig. 5), where dimensions will depend upon the character measured. Linkage

TABLE 2

The mean measurement and its variance of a character controlled by four genes whose effects are additive

Parents	Number of genotypes	Mean numerical value of character	Variance of mean
$P_1 \times P_1$	1	x	0
$P_2 \times P_2$	1	y	0
$P_1 \times P_2$ (F_1)	1	$\frac{x+y}{2}$	0
$F_1 \times F_1$ (F_2)	81	$\frac{x+y}{2}$	v
$F_1 \times P_1$	16	$\frac{1}{4}(3x+y)$	$\frac{v}{2}$
$F_1 \times P_2$	16	$\frac{1}{4}(3y+x)$	$\frac{v}{2}$

does not alter the triangular shape nor do other parental combinations such as an F_1 crossed with an F_2 segregate, or an F_1 90 per cent. selfed and 10 per cent. outbred, all have mean-values and variances which fall within the triangle. Such a situation might arise in a population undergoing active introgression.

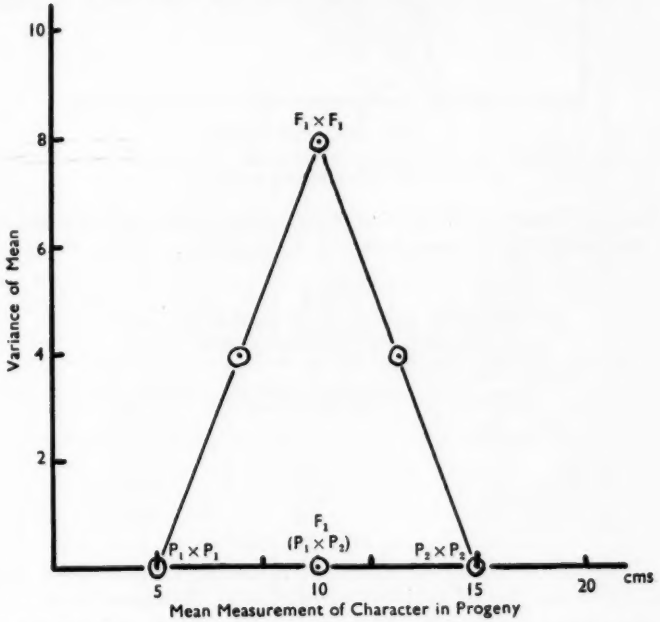

FIG. 5.—The theoretical distribution of the means and variances for a character measured on the families whose parents are given.

This theoretical hypothesis can be tested with the progeny of selfings and hybrids of species, varieties and so on, if they yield fertile hybrids, for it can be assumed for most of these that they differ principally in possessing different allelic genes. Suitable data is provided by East (1950) for cob length in maize. His numerical values are shown in table 3 and are graphed in fig. 6, from which it is seen, that for the progeny whose parents are indicated in the table, the mean and its variance for cob length mark out a triangular area.

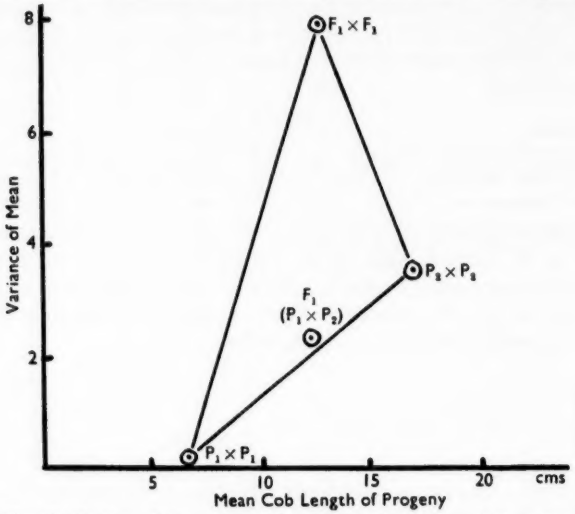

FIG. 6.—The distribution of the means and variances for cob length in maize measured on the families whose parents are given.

The application of this method of presenting data may be illustrated with the results for *E. elaeophora* and *E. goniocalyx*. When the mean

TABLE 3

The mean and its variance for cob length in the parents and two hybrid generations of maize

Parents	Mean cob length of progeny in cms.	Variance of mean
$P_1 \times P_1$	6·6	0·66
$P_2 \times P_2$	16·8	3·49
$P_1 \times P_2$ (F_1) . . .	12·1	2·28
$F_1 \times F_1$ (F_2) . . .	12·6	7·89

length-breadth ratios of the leaves from seedlings raised from each tree studied are plotted against their variances, the co-ordinates were included within a triangle. This is shown in fig. 7 which is based upon the data of table 4.

TABLE 4

The mean and variance for the length-breadth ratio of the leaves for all the seedlings raised from individual parent trees

Parent tree	Locality	Mean length-breadth ratio ± the variance at successive nodes above the cotyledons					
		1	2	3	4	5	6
E. elaeophora, typical . .	Yan Yean	3·21±0·54	2·13±0·12	1·75±0·07	1·45±0·20	1·23±0·14	1·06±0·13
	Clonbinane	3·46±0·37	2·49±0·16	2·04±0·28	1·36±0·23	1·21±0·15	1·09±0·14
	Brisbane Rge.	5·45±2·09	3·37±0·43	2·77±0·24	2·08±0·19	1·73±0·12	1·72±0·20
	Wattle Glen	4·90±0·52	3·53±0·55	2·79±0·27	2·14±0·25	1·75±0·16	1·45±0·09
E. goniocalyx, typical . .	Silver Creek	4·47±0·33	3·73±0·32	3·41±0·43	2·75±0·48	2·30±0·28	2·16±0·35
	Kinglake	4·48±0·42	3·87±0·47	3·19±0·34	2·83±0·21	2·70±0·11	2·63±0·03
	Kallista	4·48±1·87	4·21±0·49	3·49±0·14	2·83±0·44	2·69±0·41	2·37±0·34
	Mt. Wilson, N.S.W.	3·86±0·92	3·36±0·39	3·29±0·19	3·08±0·20	3·00±0·30	2·73±0·20
Intermediate between *E. elaeophora* and *E. goniocalyx* (presumed hybrids)	Reedy Creek	4·06±1·42	3·08±0·32	2·54±0·53	2·23±0·46	2·08±0·94	1·83±0·25
	Hume Vale	4·73±1·52	4·03±0·68	3·23±0·49	2·70±0·59	2·28±0·45	2·19±0·48
	Lysterfield Hills 1	4·06±1·75	3·13±0·28	2·75±0·21	2·43±0·40	2·10±0·43	1·92±0·76
	2	3·79±0·24	3·02±0·24	2·53±0·12	2·28±0·33	1·95±0·31	1·84±0·25
	3	3·30±0·18	3·08±0·39	2·58±0·22	2·13±0·44	1·90±0·40	1·70±0·67
	4	4·62±1·12	3·59±0·65	2·55±0·10	2·21±0·45	2·00±1·27	1·90±0·67

The co-ordinates within this triangle are distributed in such a way that the progeny of trees acceptable as *E. elaeophora* or *E. goniocalyx* tend to occur at the basal angles and those from the " intermediate " trees tend to occupy the centre and apex of the triangle. Such a distribution of means and variances would be expected if the " intermediate " trees were hybrid derivatives of *E. elaeophora* and *E. goniocalyx*.

When the mean-variance diagrams are compared at successive nodes it is observed that the parental trees are not similarly disposed in each ; but an analysis of the causes of this variability would be

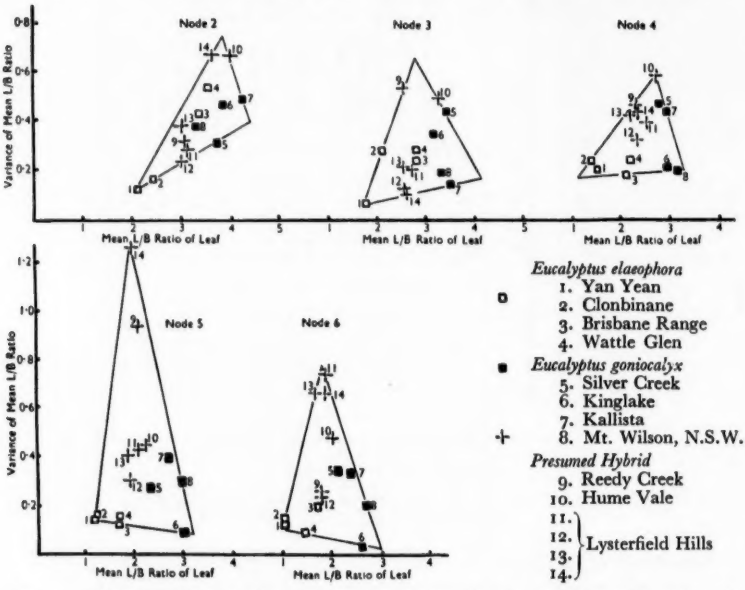

FIG. 7.—The distribution of the means and variances for the length-breadth ratios of seedling leaves in families from several *Eucalyptus* trees whose taxonomy is indicated. The data for the first node above the cotyledons are omitted as they do not conform to the general pattern.

difficult, and is better postponed until more data are available, and direct proof obtained that hybridisation is occurring in accordance with the premises underlying the theory.

3. DISCUSSION

Both the general appearance of the " intermediate " trees and their occurrence along the boundary where the habitats of *E. elaeophora* and *E. goniocalyx* meet, suggest that they might be hybrid derivatives of those species. The flowering seasons of the two species are concurrent from about February to June, so opportunity for hybridisation would exist. Furthermore, the progeny of the trees intermediate

between *E. elaeophora* and *E. goniocalyx* show segregation of characters towards these species, if leaf shape is considered (fig. 4). From the variety of leaf shapes obtained at comparable nodes of seedlings from each " intermediate " parent, it would appear that many genes control this character.

That the two species concerned can interbreed has been established with reasonable certainty from the progeny studies. No simple ratios for different leaf shape have been obtained and nothing is known concerning the extent of inbreeding under natural conditions. It is suggested, however, that the application of the " triangle " method illustrated above supplies confirmation for the hybrid origin of the " intermediate " trees and might be used to analyse other hybrid populations.

4. SUMMARY

1. Several stands of trees with characters intermediate between *E. elaeophora* and *E. goniocalyx* have been studied.

2. Segregation of characters amongst the seedlings from individual " intermediate " trees suggested that they were of hybrid origin.

3. Further support for this interpretation was afforded by field studies, which showed that " intermediate " trees occurred only in localities where *E. elaeophora* and *E. goniocalyx* grew nearby.

4. The means and variances of the length-breadth ratios of the seedling leaves for the progeny of selected trees have been used to illustrate a possible method of determining the parentage of hybrid swarms.

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LINKAGE OF LOOP-TAIL, LEADEN, SPLOTCH AND FUZZY IN THE MOUSE

GEORGE D. SNELL,* MARGARET M. DICKIE, PRISCILLA SMITH and DIANE E. KELTON

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INVESTIGATIONS by Dickie and Woolley (1950) and Fisher (1953) have established a linkage group (number thirteen) in the mouse carrying the genes *leaden* (*ln*), *fuzzy* (*fz*), *polydactyly* (*py*), and probably *Splotch* (*Sp*). The published crossover values are : *ln*—*fz*, 40·9±3·7 per cent. where the heterozygous parent is the female and 41·5± 4·5 per cent. where the heterozygous parent is the male (Dickie and Woolley, 1950) ; *fz*—*Sp*, 41·3±5·2 per cent. (Dickie and Woolley, 1950) ; *ln*—*py*, 40·3±1·77 per cent. where the heterozygous parent is the female and 23·0±2·00 per cent. where the heterozygous parent is the male (Fisher, 1953). No three-point or four-point data have been reported.

Table 1 shows the results from two sets of crosses, one involving *ln*, *Sp* and *fz* and the other *Lp* (Loop-tail : Strong and Hollander, 1949 ; Stein and Rudin, 1953), *ln* and *fz*. It will be seen that the data establish the linkage of these four genes with the order and crossover per cents. as follows :

	Lp	*ln*	*Sp*	*fz*
Heterozygous ♂ . .	35·1	5·8	31·6	
Heterozygous ♀ . .	38·1	8·5	39·8	

While these were the two most important crosses, two others gave results which deserve mention.

The *leaden Splotch* linkage was first discovered by Mrs Ann Ingalls (data previously unpublished) in a backcross of *Sp* to the V stock, a multiple-factor stock carrying *a*, *ln*, *s*, *v*, *wa*-1. The segregation of *piebald* (*s*) in this cross complicated the classification of *Sp*. However, a frequency distribution of the animals classified according to the total area of white spotting gave an indication of four modes which presumably corresponded to the four classes *spspSs*, *SpspSs*, *spspss* and *Spspss*. There was more overlapping between the *SpspSs* and the *spspss* classes than between these and the two extreme classes. The class with maximum white included animals with up to 90 per cent. of the coat unpigmented, considerably more than is ever seen in the V stock due to *piebald* alone. Evidently the effect of *Sp* and *s* in combination is more than additive. The selection of lines to divide the four classes was necessarily somewhat arbitrary ; the lines finally

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TABLE 1

Data from matings establishing the linkage and order of Lp, ln, Sp, fz. *All matings are of the backcross type*

Crosses ♀	♂	Phenotypes and number of mice								Totals	Crossover per cents.		
		ln+fz	*ln++*	*lnSpfz*	*lnSp+*	*++fz*	*+++*	*+Spfz*	*+Sp+*		*ln-Sp*	*Sp-fz*	*ln-fz*
$\frac{ln+fz}{ln+fz}$	$\frac{ln+fz}{+Sp+}$	92	44	0	3	12	3	52	107	313	5·8±2·8	31·6±2·8	35·5±2·8
$\frac{ln+fz}{+Sp+}$	$\frac{ln+fz}{ln+fz}$	42	37	1	3	9	2	30	52	176	8·5±3·8	39·8±3·8	44·9±3·8
		+lnfz	*+ln+*	*++fz*	*+++*	*Lplnfz*	*Lpln+*	*Lp+fz*	*Lp++*		*Lp-ln*	*ln-fz*	*Lp-fz*
$\frac{+ln\,fz}{+ln\,fz}$	$\frac{+ln\,fz}{Lp++}$	46	35	15	36	14	3	19	26	194	35·1±3·6	37·1±3·6	53·6±3·6
$\frac{+ln\,fz}{Lp++}$	$\frac{+ln\,fz}{+ln\,fz}$	8	9	5	4	6	1	2	7	42	38·1±7·7	40·5±7·7	50·0±7·7

selected yielded a figure for crossing over between *ln* and *Sp* of 9·7± 4·5 per cent. in heterozygous males, and 9·8±7·8 per cent. in heterozygous females.

Data indicating an apparent linkage of *Sp* and *Sd* were reported in a previous publication (Dickie, Kelton *et al.*, 1949). Since *Sd* was subsequently shown to belong in linkage group five (Wallace, 1950), some explanation of this case is necessary. The data came from matings of a single heterozygous male, with *Sp* and *Sd* in repulsion, to C57BL females. Final tabulation, including published and previously unpublished data, gave results as follows :

++	*Sp*+	+*Sd*	*SpSd*
38	64	39	29

It will be seen that the apparent linkage is due to the excess of animals in the *Sp*+ class. Only additional tests, not now contemplated, could establish definitely the reason for this unequal distribution. One possible explanation is a second spotting factor actually closely linked with *Sd*, which mimics *Sp* in phenotype and has, as *Sp* sometimes does, incomplete penetrance. *Sp* and the postulated gene are assumed to have entered the cross from one parent, *Sd* from the other.

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We are indebted to Dr L. C. Strong and Mr Willard Hollander for providing us with the Loop-tail mice. Mr Hollander also sent information that he had found no evidence of linkage between *Loop-tail* and *albino*, *pink-eye*, *short-ear*, *piebald*, *non-agouti* or *brown*.

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THE EFFECT OF THE COAT COLOUR DILUTION GENE ON BODY SIZE IN THE MOUSE

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CASTLE *et al.* (1936) state that mice with the dilution gene *d* are heavier than litter mates with the intense gene. At first they, like Green (1931), assumed that the difference in weight was due to the presence of a size gene or genes on the chromosome near the locus for dilution. In later experiments which utilised the closely linked gene for short ear *se*, they found that the marked decrease in body size of the *se* segregates could not be explained by linkage of *Se* with size genes. They and subsequent investigators postulated that the decrease in body size in short-eared mice is a pleiotropic effect of *se*. As a result of this finding it was inferred without any supporting evidence that the size effects of *d* were also pleiotropic and not the result of linkage. This untested hypothesis has been widely accepted. Castle (1940) states " The largest race of mice producible by selection should include the genetic constitution A^y *bb dd*, since this genetic aggregation would tend to increase body size over what it would otherwise be ". MacArthur (1949) in his selection experiments found that his large race were mostly *dd bb* while his small race selected from the same foundation stock were *DD BB*. He considered that this was consistent with expectation, so no test crosses or counter-selections were made to check the hypothesis of pleiotropic effects. Grüneberg (1952) is somewhat reluctant to accept Castle's hypothesis because alternative explanations are possible, such as, a size gene linked to *d* could be over-compensated by the contrary effect of *se*. In summarising, he states, " Nonetheless, taking all the pieces of evidence together, there seems little doubt that the size effects of *b*, *d*, *se*, ... are pleiotropic effects of the genes themselves."

The evidence for the effect on body size of the dilution gene is given in all Castle's later papers, and in Grüneberg's, as the percentage increase in body size caused by the dilution mutation. It is shown that dilute males are 2·30 per cent. larger than intense ones, and that dilute females are 2·47 per cent. heavier than intense females. The heterozygote is said to have no effect. It is interesting to examine the basis for these often-quoted percentages and for the table given in Grüneberg on page 407. If we utilise the given probable errors of the means to get standard errors of the differences, we find that in males the dilution gene increases body size by $0{\cdot}67 \pm 0{\cdot}27$ grams in one cross and $0{\cdot}66 \pm 0{\cdot}55$ grams in the other cross ; therefore the

2·3 per cent. increase in one cross is significant while the 2·31 per cent. in the other is not. In the females the increases are 0·49±0·19 and 1·49±0·50, therefore both the 1·86 and the 6·54 per cent. are significant. However, the large variation in the magnitude of these differences renders them suspect. In the next series of crosses the differences were smaller than those given above and were non-significant. In Green's crosses when the triple recessives *dba* were compared with *DBA*, there was no significant difference in the males but there was one in the females. When the dilution mutation was considered alone there was no significant difference in weight between the two classes of segregates.

In studying the segregation of weight in crosses involving mice with large, normal, or small body size, Butler (1952), obtained some ancillary information on the effect of coat colour genes on body size. This material was not published because it seems naïve to expect strong linkages between a major gene and genes for size, if these polygenes which bring about body size are as numerous as they appear. In view of the fact that the above mentioned hypothesis of the pleiotropic effects of *d* and *b* on body size is widely held and is in danger of being accepted at face value in modern reviews and texts, the pertinent data from these tests are given for the *d* mutant in the accompanying table. In none of the crosses was there a significant difference in body size between black and brown segregates, or between intense and dilute segregates, in spite of the fact that the segregates in one cross ranged in size from 14 to 36 grams. The table shows that in both F_2 and backcross generations the dilute segregants were smaller instead of larger than the intense ones. In the first three crosses, the *d* gene came from the parent with large body size, which was MacArthur's large (L) strain with body size of 33·6 grams. In no case is the amount by which the mean weight of the intense segregates exceeds the mean weight of the dilute ones, statistically significant, but in all cases the difference is in the same direction and opposite to what would be expected from pleiotropy. While these results are contrary to those expected on the hypothesis that one pleiotropic effect of *d* is to increase body size, it can be argued that the expected pleiotropic effect is so small that segregation of other size genes could obscure it. To eliminate this criticism we need to observe the size affects of *D* and *d* when the rest of the genotypic background is identical. A segregating inbred line is ideal for this purpose, and the lower half of the table gives data from such a line.

The *pbs* line was set up in 1949 from selected segregates of a cross between McGill and Toronto strains. The line was established by brother ×sister mating and in the first generations the only selection applied was for general vigour. After Sir R. A. Fisher had visited our laboratory and pointed out the superiority of segregating inbred lines over standard inbred ones, we decided to maintain some of our

stocks in this form. Since the *pbs* line was still heterozygous for *Dd* we mated an intense brother with his dilute sisters, and vice versa in all generations after the eighth. The line is fairly vigorous although there has been some infertility and a decline in weight, from 22·1 for males and 20·3 for females, to the values given in the table. The data in the table show that in this inbred line, with its fairly uniform

TABLE

Sixty-day weights for intense and dilute mice

Cross	Type	Males		Females	
		Number	Mean	Number	Mean
(L×N) F_2	*D*	88	28·1±0·38	90	23·8±0·31
	d	33	27·1±0·48	24	22·9±0·39
	D-d		1·0±0·61		0·9±0·48
(L×N) B.C.	*D*	43	31·6±0·36	40	26·5±0·38
	d	45	31·2±0·36	35	25·9±0·39
	D-d		0·4±0·51		0·6±0·54
(L×*pbs*) F_2	*D*	102	26·7±0·36	82	23·4±0·52
	d	43	26·3±0·51	29	22·4±0·55
	D-d		0·4±0·63		1·0±0·76
Inbred pbs generations 9-11	*D*	21	21·03±0·58	39	18·27±0·68
	d	43	19·73±0·36	23	17·76±0·63
	D-d		0·30±0·68		0·51±0·93
generations 12-14	*D*	24	19·05±0·70	38	16·24±0·40
	d	27	17·41±0·54	26	15·68±0·56
	D-d		1·64±0·88		0·56±0·68
generations 15-17	*D*	26	18·66±0·42	14	16·79±0·54
	d	12	18·01±1·00	17	16·16±0·46
	D-d		0·65±1·08		0·63±0·71
All generations	*D*	71	19·67±0·35	91	17·20±0·33
	d	82	18·83±0·30	66	16·53±0·35
	D-d		0·84±0·46		0·67±0·49

genic background, there is no evidence for the suggested pleiotropic effect of *d*. In fact, while none of the differences are significant, they are all in the opposite direction to that expected. In the table, because of the small numbers involved, three generations are lumped together to give each set of data, but this lumping did not change the picture. When comparisons are made within each generation we find that of the 18 differences found, in only two cases were the

dilute segregates heavier than the intense ones, so it appears that while the differences between the two types of segregates are not significant in any single case, the very homogeneity of these differences points to the fact that the differences are real ones rather than chance ones. The segregation ratios for *Dd* are quite good in the case of the F_2's and backcross but are not so consistent in the inbred line. It does not seem necessary at this time to justify this departure from the 1 : 1 ratio or its apparent association with sex.

The interpretation of these findings is that dilute does not have a pleiotropic effect on body size. If it did exhibit this pleiotropism it would be necessary to postulate in this case that linked with *D* there was a gene or genes which increase body size, and that the effect of these genes overrides the pleiotropic effect of *d*. The law of parsimony causes us to abandon this explanation, especially since the pleiotropic effects of *d* were not well substantiated in the first place. The two alternative explanations are either the weight differences between the two types of segregates are chance ones and no explanation is needed, or if we accept as a criteria of a small real difference the fact that the weight differentials are all in the same direction ; then we have to postulate linkage. Such linkage must consist of size genes close enough to the *D* locus that after 17 generations of inbreeding they are still on the tract of heterogeneous origin (Fisher, 1949). The length of this tract if of the enclosed type, would be between 4·5 and 5 centimorgans for the 16th and 17th generations of inbreeding. Further information on this point should accumulate in future generations of inbreeding when the decrease in the length of this tract will make body size genes, if they do occur in this region, homozygous. If the hypothesis of size genes linked to *D* is accepted, then the earlier work of Castle fits into the same scheme, except that in his case the linkage was in the opposite phase. The percentage increase in body size of the *d* segregates in his data is comparable with the percentage decreases in the present data.

Summing up, it seems advisable to abandon the hypothesis that the dilution gene has a pleiotropic effect on body size, and to substitute in its place the hypothesis that close to the *d* locus there are genes which affect body size.

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REVIEWS

THE FACTS OF LIFE, by C. D. Darlington, 467 pp. (Illustrated). George Allen and Unwin, London, 1953. 35/- net.*

In the first half of the twentieth century biology uncovered a series of facts about the general nature of living organisms which do not fit in easily to the picture which earlier generations had formed. It is not simply that the older ideas included a few errors; the point is that the new wine has fermented and split the old bottles, and that we have to make ourselves a new system of concepts, a new way of looking at living things, if we are to arrange our knowledge in an orderly way and use it to the best advantage.

It is the scientific study of biological inheritance—the science of genetics—which has been mainly responsible for the fundamental new advances. The critical point is this. Previous civilisations, and particularly the mediæval out of which we ourselves have developed, conceived of the basic nature of man as something insubstantial—a soul, or an essence, a set of "humours". And further, this essence was not thought of as causally effective, it did not react with anything and produce an effect, it simply stayed itself, the quintessence, the ultimate reality of the man. When human beings or other animals reproduced, it was the essences of the two lines of ancestors which came together from the mother and father and blended in the offspring. Heredity was spoken of in terms of "blood"; not the real red fluid, but a semi-mystical essence. Now the scientific study of living things has concentrated on the process of reproduction, because it is at the very beginning of the life of an individual that we can examine it when it is reduced to its simplest terms. As Darlington puts it, the older theories suggest that we ought to find that "the reproductive essence or material, the germ or seed, is a contraction or condensation or abbreviation of the parent at the moment of begetting, a contraction which expands or unfolds or grows by a reverse process to give the mature offspring". What we actually find is something quite different; not humours or essences, but a set of material particles, each with specific properties which cause them to interact in definite ways with each other and with their surroundings and thus gradually bring the new creature to maturity. Our concepts have to leave the world of Essence and Being, for the hard unpalatable realm of Matter, of cause and effect, and Becoming.

In the first two sections of his book, Darlington traces the stages of this journey. The first break with the old ideas came with the development of the cell theory early in the last century. Then followed three steps overlapping in time and to some extent confusing each other: Darwin's insistence that evolution is the guiding principle of biology, and natural selection the mechanism of evolutionary change: Weismann's argument that there is a fundamental distinction between the body as a whole and what he called the Germ-plasm, which is the only part of the living organism which is passed on in heredity; and the discovery of the cell nucleus and the chromosomes which it contains. It was only after these advances had

* This review was originally written as a talk given in the Third Programme of the British Broadcasting Corporation.

prepared the ground that biologists could assimilate the most important discovery of all, that of the existence of discrete, more or less unchanging hereditary factors, though Mendel had made it more than thirty years earlier. In the first two decades of this century, William Bateson in this country, and Morgan, Muller, Bridges and Sturtevant in America, worked out in detail the behaviour of these factors, or genes as they came to be called, and firmly established a science of genetics. But the edifice was not complete until the material basis for this behaviour had been made clear by the study of the chromosomes which carry them. In this final phase, in which genetics became transformed into cytogenetics, Darlington's own contribution has, of course, been of the greatest importance.

Darlington's views on the import of genetics for the general problems of civilisation are based on many years of remarkably broad and productive experience ; and they all deserve very serious consideration. I do not agree with all of them. Darlington's main point is that each man is born, not as one more exemplification of a universal, equally distributed human essence, but with a particular outfit of genes, which endow him with certain specific potentialities, and certain limitations ; in fact, with an individuality. And Darlington himself is a text-book example. How refreshing it is to read a scientific book in which there is scarcely a point which anyone else would have seen in quite the same way. Many of Darlington's shifts from the accepted conventional emphasis are extremely stimulating ; there are many, of course, on which I should like to argue with him ; there are some in which I think he is definitely wrong. There is not time here to go into all these details. I will mention only one place where I think that the balance of his historical survey is unsatisfactory ; he seems to me to overlook the importance, in the endeavours to establish a causal conception of biology, of the German experimental embryologists such as Roux and later Spemann. And one point about his exposition of scientific theory. When he descends to what he calls the underworld of genetics—the murky realms of viruses, plasmagenes, cancer-producing agents and so on, with which experiment is only just beginning to grapple successfully—in my view he should be followed only with caution and keeping one's fingers crossed.

The most important part of the book is the latter half, where Darlington presses boldly on to consider the implications of the new biological outlook. The range of topics he touches on is very wide, as is indicated by the titles of his last two sections, Man and Woman, Thinking and Knowing. He deals—and interestingly too—with such a hoary old topic as free-will. He offers some suggestions about the evolution of Art, but on this I think Herbert Read has carried the same train of thought further and made it more convincing by doing so, (*Art and the Evolution of Man*, Freedom Press, 1951.) He criticises Freud, and Marx, and the modern advocates of the view that we must give up our old ideas of determinism. And these old problems or modern trends of thought are confronted, not with a few simple biological commonplaces, but with an exceedingly subtle and penetrating system of ideas.

Modern biology, in the hands of those who know it well and widely, does not operate in terms of simple chains of cause and effect. We soon find, if we try to think of A as a cause which has the effect B, that B also has an effect on A. For instance, the environment selects the type of animal

which fits it best; but at the same time, animals will select, out of the range available to them, the environment in which they choose to live. We have to deal in biology with a network of reciprocal interactions, not with the simple causal sequences of conventional logic. Again, we always have to remember that we are dealing with things which are organised, as it were, on a series of different levels, each of which has its appropriate laws. In the study of evolution for instance, one level of organisation is that of the relations, of eater and eaten, hunter and prey, between the various species of animals and plants which co-exist in any one place. That system has its own laws. Quite different laws govern the lower level of organisation in which genes and environment conspire to produce the adult animals. Different again are the laws of the genes themselves, in their transmission from one generation to the next and in their stability through the lapse of time.

Darlington's thought is thoroughly permeated by this specifically biological outlook. He uses it with particular penetration, I think, in his discussion of the recent suggestions that we ought to give up our old idea that the world is based on determinism and suppose instead that scientific laws are fundamentally statistical. He distinguishes, first, between "indeterminism"—a situation in which there really is no direct connection between cause and effect—and "uncertainty" where we merely do not know enough to be able to work out the connection. He doubts the validity of indeterminism. But he shows how life employs uncertainty at one level, that of the gene and its changes, to bring about a more definite deterministic relation than would otherwise be possible at a higher level, that of the organism and the conditions of its life to which it becomes adapted. "This intercalation of uncertainty," he says "has become the key mechanism of evolution. It is perhaps the fundamental discovery of life."

But although Darlington knows well how the laws of one level of organisation may override those of lower levels, the main criticism I have to make of his book is that he does not take this into account in a context where it seems to me fundamental. That is in relation to man. Darlington stresses what I should like to call the hard facts of life—the fact that people are not born with equal potentialities, but with capacities which inescapably depend on the genes they received from their parents. That is true, and it must be faced without sentimentality or evasion; but equally there is no reason to assume the worst unless we are forced to. Just how much latitude do the genes allow? Granted that at his conception a person is provided with certain limited potentialities, how much can the realisation of those potentialities be altered by the circumstances under which he is brought up? Possibly, within any one society it is less than we might hope. Darlington certainly thinks so. "Economic circumstances", he says "alter the expression of virtues as well as vices. But in themselves they play little part in creating them". And "how often do we find that the sons of great men are given the best chances, with the worst results".

Darlington also argues, and I think quite correctly, that there are great genetic differences between the various races of mankind—differences affecting not merely trivial characters such as skin colour, but the basic abilities for the manifold and varied activities of living. This conclusion is often evaded by well-meaning people who think that it will provide an

intellectual justification for racial persecution. But Darlington suggests that its real import is just the opposite and that genetic differentiation of local populations is favourable to evolutionary advance. " The assumption of a genetic basis for race and class differences," he says, " provides the evidence, the only scientific evidence, in favour of racial tolerance and co-operation. . . . The future rests with those genetically diverse groups, whether races or classes, which can practise mutual help and show mutual respect."

These are the hard facts of life. I call them hard for two reasons. They are hard in the sense that they are only a little way removed from solid and careful experiments ; and hard also because they emphasise the limitations which the genetic constitution imposes on human development. But I think there is another group of facts, which I might call soft facts, to which Darlington pays too little attention. They are soft both because their experimental basis is not so clear cut, and because their implications are kinder to our idealism. They are the facts about the social mechanism of transmitting ideas and practices from one generation to the next. Man has in fact developed a completely new type of heredity, which enables him to pass on to later generations the ability, say, to fly, by a means in which genes are not *directly* concerned at all, that is to say by education. This is a process at a higher level of organisation than the genetic mechanisms of animal evolution. It must have its own laws. A new gene persists in evolution if it is favoured by natural selection. What decides whether a new concept or idea will persist ? We simply do not know. Certainly it is not its sheer usefulness to mankind, or we should have had universal peace for many centuries by now. But there is here, I think, a whole science waiting to be worked out, as complicated as that of animal evolution, and for human affairs even more important. Particularly if we try to compare human races, it seems impossible to make much progress until we can assess the importance of their different cultural traditions in determining how their genetic potentialities become realised. My own opinion is that up to the present we know next to nothing about the whole subject ; and that all we can usefully say about races is that they almost certainly must be genetically different, that it is probably a good thing that they should be, but that we have no idea how different they are or what the differences consist in.

The vigour of Darlington's thought, and prose, and the combination of a highly individual point of view with a wide field of scientific knowledge, will, I think, cause this book to be quoted for a long time as an important mid-twentieth century opinion about the cultural influence of biology.

C. H. WADDINGTON.

Rh-Hr BLOOD TYPES. Applications in Clinical and Legal Medicine and Anthropology. Selected Articles in Immunohematology. By Alexander S. Wiener, M.D., F.A.C.P., New York : Grune and Stratton. 1954. Pp. 763. $11.50.

In the fifteen years since the discovery of the Rh groups there has been continuous progress in our knowledge of their serology, genetics and applications. The field has grown to be large and complex, so that there must be many people who would welcome an authoritative monograph on the Rh groups. The scope is indicated in the second subtitle : the volume comprises, in fact, a selection of 82 papers published by Dr Wiener

and his colleagues and two papers published by Dr Wexler in support of Wiener's contentions, together with notes on technique and a bibliography listing 333 papers published by Wiener in the years 1929 to 1953.

The declared objective of the anthology appears in the preface: "It seemed worthwhile to collect in one volume the author's most representative and important contributions to this subject, thus giving an idea of how knowledge in this field developed."

This bold identification of author with subject sets the tenor of the whole volume. None would wish to deny the importance of Dr Wiener's contributions to the serology of the Rh groups. The discovery of the Rh groups by Landsteiner and the author; the demonstration with Peters of Rh immunisation as a cause of hæmolytic transfusion reactions; the pioneer work on "blocking" or "incomplete" antibodies and their placental permeability; the applications of these findings to hæmolytic disease of the newborn and forensic medicine: writings on all these subjects are included, and they show the power and versatility of Wiener's mind and the competence with which he can handle difficult material.

Nevertheless, it scarcely needs to be pointed out that others have tilled the same field and have reaped results of comparable importance. The reader will seek in vain a reasoned account and criticism of the work of Levine, Fisher, Race and Mourant, not to mention others. Wiener's references to other authorities are usually grudging, sometimes openly hostile. There is evidence throughout the volume of a tendency to rake over the ashes and rekindle the dying embers of a controversy long since grown wearisome. The controversy concerns, of course, the Rh nomenclature; Dr Wiener repeatedly stresses the advantages of his own notation and the drawbacks, even absurdities, of the CDEF notation.

"Controversy" is hardly the word. Dr Wiener has been recognised these many years as the champion of the Rh-Hr notation, and it seems that he is still poised, pen in hand, ready to do battle at any time and place. The protagonists of the CDEF notation, however, have been unwilling to enter the lists. They have relied, rather, on the arts of peace and gentle persuasion, and they have confidently let their notation plead for itself. One is forced to conclude from Dr Wiener's protestations, that the CDEF notation is gaining general support. There must be few geneticists to-day who doubt the soundness of the theoretical foundation of Fisher's and Race's hypothesis and the consistency and essential correctness of the genetical interpretation which has been developed upon it. This construction is a good deal more substantial than Don Quixote's windmills; small wonder that to tilt against it is discomfiting.

Dr Wiener's conduct sometimes seems unchivalrous. The tone of his asides can be judged from this quotation (p. 364): "I.M. Jaundiced, a poet residing at 36 Genotype St., High Titer, R.H., has celebrated the discovery of little f by composing a song entitled, 'C, D, E, F, . . . Gee!'" Moreover, he uses the symbol F for the Duffy antigen, which had previously, and with genetical precision, been designated Fy^a, and he supports an earlier suggestion that the symbol C should refer to an antigen of the ABO complex. The adoption of these terms can hardly have had any purpose other than that of introducing confusion into the CDEF camp.

The arguments for and against each of the two notations need not be recapitulated here. To the pathologist it is of very little practical importance

whether there is in the Rh groups a complicated system of multiple allelomorphs or a series of four closely linked factors. What the pathologist requires is a system of nomenclature that is easy to learn and to use. It is, of course, possible to master almost any system of notation, just as it is possible to master the cuneiform script and the Boolean algebra of symbolic logic. However, we are concerned not with intellectual gymnastics but with a system which is rational and easily workable in practice. Even consistency and genetical precision can justifiably be sacrificed : the use of the symbols ABO to designate allelomorphs as well as antigens already defies current genetical practice. To the reviewer it seems that there may be real advantages in the retention of a duplicate system of notation for this purpose. Thus, the Rh notation allows concise designation of the phenotypes of the antigens and also of the corresponding chromosomes. But the CDEF notation undoubtedly simplifies the genetical interpretation, thereby rendering the whole system easier to learn and remember, and it allows concise designation of antibodies. Once a system of nomenclature is laid down it should be adhered to and not tampered with year by year, as Wiener has shown a tendency to do with the Rh notation. The difficult question of a convenient terminology will not be decided here and now, in any case ; it will be resolved only by time and future custom.

On the other hand, to the geneticist and the evolutionist who is interested in the different ways in which particular genetic situations can arise and establish themselves in natural populations, the distinction between linked factors and multiple allelomorphs is of the greatest importance. The utmost possible precision in notation is also essential, and there is little doubt that this is better supplied by the symbols of Fisher and Race than by those of Wiener. The question of priority need not be laboured : in genetics the best notation has precedence over the earliest notation, thereby avoiding the stultifying effects which strict adherence to priority has sometimes produced in taxonomy. Even in the Fisher-Race system of notation, there should be separate and always distinct terms for genes, antigens and antibodies. The letters themselves, ' C, c, . . . " should refer to the genes concerned, the corresponding antigens should be designated " C-antigen, c-antigen . . . . " and the antibody can be conveniently termed, as it always has been, " anti-C " or " anti-c ".

As far as polemic is concerned, the lesson of the past is plain for all to read. The history of science includes a succession of acrimonious disputes, and it can be said with confidence that the reputation of the disputants invariably suffered, the advancement of knowledge often being held up for a generation or longer. Thus, the haggling of Newton and his followers with Leibnitz and his followers over priority in the discovery of the infinitesimal calculus was damaging to the characters of both great men. And the obstinate British were almost barren mathematically during the succeeding century while the more progressive Swiss and French perfected the calculus and made it the simple, easily applied implement of research that Newton's successors should have had the honour of making it. Moreover, Newton's dispute with Huyghens over the nature of light might in happier circumstances have been resolved so as to show that there was right on both sides, thereby opening the way to the unified corpuscular and undulatory theory which was developed only very much later. There is, again, the case of Priestley who adhered to the Phlogiston hypothesis

even after his own experiments had made it untenable. Not convinced by the brilliant researches of Lavoisier, Priestley remarked, " I have well considered all that my opponents have advanced, and I feel perfectly confident of the ground I stand upon. . . . Though nearly alone I am under no apprehension of defeat."

Compare these unfortunate incidents with the honourable solution of the question of priority in the recognition of natural selection as an agent bringing about evolutionary changes. Lyell and Hooker sent to the Secretary of the Linnaean Society the following letter, referring to Darwin and Wallace : " These gentlemen having, independently and unknown to one another, conceived the same very ingenious theory to account for the appearance and perpetuation of varieties and of specific forms on our planet, may both fairly claim the merit of being original thinkers in this important line of enquiry ; but neither of them having published their views, though Mr Darwin has for many years past been repeatedly urged by us to do so, and both authors having unreservedly placed their papers in our hands, we think it would best promote the interests of science that a selection of them should be laid before the Linnaean Society."

Again, Pasteur, having shown that optically active organic compounds rotate the plane of polarised light even when in solution, drew the bold, and, as it turned out, correct conclusion that the molecules of such compounds are themselves asymmetrical. This led him into conflict with Biot, who was not without doubts regarding the accuracy of Pasteur's observations. Biot called Pasteur before the Academy, handed to him some racemic tartaric acid, soda and ammonia, and bade him repeat the experiments. The crystals prepared, Biot himself made the solutions and examined them in the polarising apparatus. Then, in Pasteur's own words, " Without even making a measurement, he saw by the appearance of the tints of the two images, ordinary and extraordinary, that there was a strong deviation to the left. Then, very visibly affected, the illustrious old man took me by the arm and said, ' My dear child, I have loved science so much all my life that this makes my heart throb.' "

All things considered, *Rh-Hr Blood Types* is at once an example and a cautionary tale. The example appears in the speed and deftness with which a seemingly trivial observation (that a rabbit antiserum against monkey red cells agglutinates some but not all human cells) was exploited in the brilliant serological discoveries concerning transfusion reactions and *erythroblastosis fœtalis*. The other side appears in Wiener's reluctance to recognise the profound significance of the work of others in the field which he has come to regard as his own preserve. There are no monopolies in science.

ANTHONY ALLISON.

AN Rh-Hr SYLLABUS. The Types and Their Applications. By Alexander S. Wiener, M.D., F.A.C.P., F.C.A.P. New York : Grune and Stratton. 1954. Pp. 82. $3.75.

The purpose of this booklet is to present an up-to-date summary of the Rh groups and their applications to clinical and forensic medicine and anthropology in a compact, easily understandable form. It is intended as an introduction : " for readers not specialising in the field, it contains all the information they require and will make it possible for them to read and understand without difficulty current articles on the subject,

no matter how complex." The material has been arranged in the form of a well-organised glossary, which makes for easy reading. In general, the presentation is clear and sound. It is at times perhaps unnecessarily dogmatic : thus, there are some who would take exception to Wiener's statement, " Reports that pregnancies even with Rh-negative babies may cause a non-specific anamnestic rise in titer (of Rh antibodies) are erroneous." The term " conglutination " is retained for the albumin agglutination technique in spite of the fact that conglutination has long been used in quite another sense in serology.

The booklet is marred by the trumpet blasts which are sounded *fortissimo* against the CDEF notation and theory of linked factors. None the less, the theory and the notation, unlike the walls of ancient Jericho, still stand, and they are of great value to the student who is being initiated into the problems presented by the Rh groups. In any case, a proper understanding of the CDEF terminology is necessary for the interpretation of current papers on the subject. ANTHONY ALLISON.

ANIMAL SPECIES AND THEIR EVOLUTION. By A. J. Cain. London : Hutchinson's University Library. 1954. Pp. 190. 8s. 6d.

Until about two generations ago, systematics and descriptive morphology were predominant in biological research and in academic curricula. Owing to the spectacular development of experimental biology, the pendulum of popularity has swung far away from systematics. At least in the United States, theses based on research in systematics are not regarded in many universities as satisfying the requirements for the doctor degree. Some of the major foundations show a disinclination to support research in systematics, while being very generous to other branches of biology. Since the war, however, signs are multiplying which suggest that the anti-systematics bias is also passing. The current of the so-called " new systematics " has definitely gathered strength, and systematics seems to become once more integrated with other biological disciplines in a broad synthesis which is now emerging in modern biology. Perhaps this re-integration is being brought about not so much by new systematics as by new systematists, who combine a wide knowledge of the variety of living things with an insight into the problems of general biology and a thorough understanding of the scientific method. Dr A. J. Cain is one of these new systematists, and his compact but forceful book should be equally useful to systematists and to other biologists as a prophylactic against mutual prejudice and short-sightedness.

Dr Cain has devoted the first five chapters of his book to a brief exposition of the scope and of the working methods of taxonomy. The presentation is concise and admirably clear. The illustrative examples are well chosen to elucidate the ideas discussed, although these examples often deal with animals which inhabit remote lands and which will be rather unfamiliar to a majority of the probable readers. The sixth (" The Biological Species ") and the eighth (" Geographical Speciation ") chapters are perhaps the most important, and also the best written, in the book. The last chapter (" Sympatric Speciation ") deals with some more specialised topics.

The species of classical taxonomy are morphological species, or " morpho-species " according to Cain. Morphospecies are defined entirely on morphological characters ; some taxonomists still persist in refusing to

consider even the possibility that species can be defined in any other way. Morphospecies are static and unchangeable in space and in time, because they are essentially names attached to a single " type " specimen or to a small series of specimens. But Cain rightly points out that morphospecies continue to be useful in modern biology. They epitomise the results of the first stage of investigation of biological materials, which consist frequently of isolated specimens, often imperfectly preserved, of little known animals and plants from little known lands and seas. An orientation in the biological materials is needed before a deeper study is undertaken. Before all else, biologists must know what they are talking and writing about. But the first stage of investigation should not be also the last. Morphospecies is an inference of biological species. Biological species are not names and not specimens. They are Mendelian populations, or groups of populations, reproductively isolated from other populations or groups of populations.

In 1935 this reviewer proposed a definition of biological species embodying the above principles ; this definition seemed then far-fetched to many taxonomists and even to some geneticists, since it required that certain organisms which are similar morphologically must nevertheless be considered belonging to distinct species. For example, the so-called A and B " races " became two species, *Drosophila pseudoobscura* and *Drosophila persimilis*, because they maintain their integrity in nature when living side by side in the same territory. Much water has flowed under the bridges since 1935, and the existence of some morphologically similar but reproductively isolated " sibling " species is no longer shocking to most systematists. As stated by Cain, " There is no reason why the techniques commonly employed in museums up to now should be all-sufficient for detecting the limits of species. One might as well ask all chemists to use only the apparatus known to John Dalton, since he was able with it to produce the atomic theory."

Like so much else in biology, the phenomenon of species acquires its full meaning only when considered in the light of evolutionism. Cain gives a very lucid and concise account of the modern theories of origin and maintenance of species. Geographic differentiation of a species into spatially segregated races is recognised as the usual precursor of the splitting of an ancestral species into two or more derived ones. Races may diverge and become incipient species. Sympatric speciation (splitting of a Mendelian population into two or more populations inhabiting the same geographic area) may also occur under certain exceptional circumstances, but it is probably rare compared to geographic (allopatric) speciation. Adaptation to environmental diversity by means of natural selection is rightly recognised as the principal driving force of race and species formation and of evolution in general. Some arguments used to demonstrate this point are, unfortunately, overstated. Thus, the existence of stable geographical gradients (clines) in many traits of many species is said to be evidence of the operation of natural selection in race formation. Now such gradients probably are due to selection in most instances, but to say that they prove selection is a specimen of circular argument. So, one may well apply to the author his own words applied by him to Charles Darwin : " The cause of his error was only over-appreciation of certain truths." THEODOSIUS DOBZHANSKY.

HEREDITY IN HEALTH AND MENTAL DISORDER. By F. J. Kallmann. New York: W. W. Norton & Co. Inc. 1953. Pp. 278. $6.00.

A number of detailed and thorough accounts of the genetics of mental disorder have recently appeared. The present work is by Dr F. J. Kallmann, a pioneer in the twin method of psychiatric research. It differs from its predecessors in being addressed to the body of psychiatrists who are ignorant of genetics but wish to find out what it has to do with their work. It consists of three lectures, the first two of which are illustrated by 89 plates. These are mostly photographs of normal and abnormal one-egg and two-egg twins, illustrating very usefully the degrees of physical concordance which are so closely related to degrees of mental concordance.

The data contained in the tables and text are of great interest, greater even than the author realises. Why he fails to realise it is one of the things that the text of the book makes clear. Dr Kallmann does not seem to understand that the study of one-egg twins can be used to discover the mode of action of the environment as well as of heredity. He does not comment on the effects of child-bearing so strikingly demonstrated in his data. Of the mode of action of heredity he is also not much clearer than his readers are likely to be. He writes: "It is to be expected that the range of differences between two-egg twins will somehow depend on the extent of dissimilarities between their parents (p. 70)". But surely it is the *grand*parents that matter in this respect. And *somehow* is mendelian segregation.

The arrangement and detail of this book also introduce confusion for the readers. The extensive discussion of resistance to tuberculosis (pp. 152, 155-164) in the sub-section labelled "schizophrenia" is quite inconclusive. The adding together of the concordance columns for one-egg and two-egg twins (table 9) is a shocking example to beginners. The frequent references in brackets in the text to *nomina nuda* like "Dr Planansky" (p. 162) whose names do not appear in the already enormous bibliography adds to the impression of muddle.

The methods used by Dr Kallmann are based on sound genetic principles and their exposition is greatly needed in medical training. But if the exposition is not rigorously connected with genetic principles and the principles themselves are not understood, who can tell what strange harvest we may reap?

C. D. DARLINGTON.

INTRODUCTION TO MALTHUS. Edited by D. V. Glass. London: C. A. Watts & Co. 1953. Pp. 205. 10s. 6d.

In his little politico-economic pamphlet on population published in 1798, Malthus advanced a revolutionary argument based not on moral but on biological principles. One of these was the explicit principle that the human population of our planet must be controlled either by premature death or by the prevention of birth since it cannot increase without limit. The other was the implicit principle that men differed by heredity in their social habits and capacities in a way affecting their choice of these alternatives and their capacity for avoiding them especially through the ownership of property.

The first of these principles has influenced all later discussions of population and of economics; the two combined after 1858 became the foundation of evolutionary theory. But the theories of population and of

economics have in general omitted the second. This is partly because the later and weightier editions of Malthus' essay, like the later and weightier editions of Darwin's *Origin of Species*, change the emphasis of his argument. It is partly also no doubt because the principles of economics become unmanageable if an additional genetic variable is introduced into the theory. The omission however is a serious one because it conceals the contradiction which economists and sociologists are unwilling to admit, the contradiction between the economic and the biological aspects of Malthusian theory, the one demanding a standardisation of individuals, the other rejecting any standardisation of individuals.

The present book brings this contradiction home to us very well. For it is an attempt to recall the work of Malthus to our memory to-day. It consists of three accounts of thought on the subject in the eighteenth, nineteenth and twentieth centuries, a bibliography of Malthus and Malthusianism from 1793 to 1880 and reprints of Malthus' Summary of 1830 and his letter to Samuel Whitbread of 1807. The book as a whole is bound to be of great value. It has only one grave flaw : the failure to recognise the other side of the Malthusian system. The book as it stands is interesting. If this flaw were repaired it would be more than interesting. It would be gravely disturbing. C. D. DARLINGTON.

IDEOLOGIE UND FORSCHUNG IN DER SOWJETISCHEN NATURWISSENSCHAFT. By A. Buchholz. Stuttgart : Deutsche Verlags-Anstalt. 1953. Pp. 126. DM 2.90.

A review of Russian scientific literature from the end of the war to the death of Stalin. It deals with all branches of science, with the theories of Butlerov, Oparin, Lepeschinkaya and Lysenko and with the myths now attached to the names of Timiriazev, Michurin and Pavlov. There is an historical introduction and a bibliography of 376 papers, mostly Russian.

BIBLIOGRAPHY ON THE GENETICS OF DROSOPHILA. Part 2. By I. H. Herskowitz. Commonwealth Bureau of Animal Breeding and Genetics. 1953. Pp. 212+xi. 21s.

Part 1 of *Bibliography on the Genetics of Drosophila* was prepared by Professor H. J. Muller and included papers up to 1938. Dr Herskowitz has now brought the tally up to 1950 in Part 2, which, like its predecessor, is published by the Commonwealth Bureau of Animal Breeding and Genetics.

The new volume includes 2841 books and papers and an index in two parts. The publications are arranged alphabetically by the name of the senior author, with full cross-references against the names of junior authors, so that a title can be readily traced once the name of any one of its authors is established. At the same time the General Index, occupying 40 pages and including some 1700 headings, should make it easy to trace papers by the subjects with which they deal. A shorter Systematic Index classifies the papers primarily by the species of *Drosophila* to which they refer. The index covers Part 1 as well as Part 2 of the Bibliography and so makes good a deficiency of the earlier publication. As in Part 1, titles originally given in a language other than those of Western Europe have been translated, and authors' names have been transliterated using anglicised orthography.

Publications have not been chosen for inclusion in any restrictive way. The systematics, morphology, physiology and natural history of *Drosophila* are included as well as the genetics. Publications not primarily concerned

with *Drosophila* have also been brought in where they would seem to be appropriate, so that many genetical books and reviews of a general kind are to be found in the list. By including articles from Drosophila Information Service, Dr Herskowitz has ensured the availability of a great deal of technical information not published in the normal way. He has taken the opportunity of catching a few publications which had eluded Professor Muller when he was preparing the earlier part.

Although a bibliography is not designed primarily to record the progress of a science, a great deal of information can in fact be gained from it. A striking feature brought out by Dr Herskowitz' publication, and one to which Professor Muller draws attention in his preface, is that the average annual output of papers relevant to *Drosophila* genetics has increased from 173 per year in the period 1925-38 to 237 in the years 1939-50 ; and this despite the facts that *Drosophila* has lost something of its dominating position in genetics and that the period in question includes the war years. Such figures bring home to us the accelerating progress of genetics itself as reflected in its ever-increasing literature ; and at the same time we can see by the general references, which it is found desirable to include in a *Drosophila* list, how genetics is maintaining and even strengthening its unity as a scientific discipline, despite the growing spread of the living material from which it draws its experimental evidence. We can see, too, a different and more melancholy kind of history recorded in the pages of this bibliography when we observe the dates at which the publications of geneticists such as Dubinin and Timoféeff-Ressovsky suddenly cease.

But it is primarily as a guide to an extensive and essential genetical literature that *Bibliography on the Genetics of Drosophila* must be judged. It will fulfil this purpose admirably, and indeed it will be a necessity to every laboratory where genetics is practised. K. MATHER.

THE PLACE OF BOTANY IN THE LIFE OF A UNIVERSITY. Inaugural Lecture. By C. D. Darlington. Oxford : Clarendon Press. 1954. Pp. 24. 2s. 6d.

An attempt to show that the development of analytical methods in the study of plants, chemical, genetica land microscopical, which was frustrated in the seventeenth and eighteenth centuries, has brought botany into relation with the other sciences and with the humanities, and thus requires the breaking down of the antique barriers between departments.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND FOURTEENTH MEETING of the Society, held on MONDAY, 5th APRIL 1954, at the GALTON LABORATORY, University College, Gower Street, London

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THE DEVELOPMENT AND ANATOMY OF THE MUTANTS VARITINT-WADDLER, JERKER, AND SHAKER-2 IN THE MOUSE

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All three genes affect the labyrinth in essentially the same way. There is no gross abnormality in the organ and the degenerative changes set in when the histological differentiation is nearly complete. There is dedifferentiation of the organ of Corti and degeneration of the spiral ganglion, the stria vascularis, the maculæ and the cristæ. In the vestibular ganglion the average cell-size is considerably reduced. However, the three mutants differ in the time of onset of the anomaly in different parts of the labyrinth ; also, the cristæ are apparently normal in shaker-2 and jerker and the vestibular ganglion in jerker.

GENETIC AND ENVIRONMENTAL INFLUENCES ON SEX RATIO IN MICE

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Over 4500 mice were bred from four inbred strains and from the 12 possible crosses between them. Mice were sexed within 24 hours of birth. Effects on the sex ratio of genotype, litter size, and parity are analysed and discussed.

SELECTIVE ADVANTAGE OF HETEROZYGOSIS FOR BLOOD GROUP GENES AMONG INBRED CHICKENS

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In a line of White Leghorn chickens which has been maintained by brother-sister matings for fifteen generations, segregation is still occurring at three independent loci determining antigens of the red blood cells. Approximately 50 per cent. heterozygosis for each locus occurs in each generation.

Artificial selection has involved the retention as breeders of individuals from the largest sib-groups in each year. In this selection individuals with three or two blood group loci heterozygous have an advantage over those with one or no heterozygous loci. This is shown in two ways. Firstly, the more heterozygous dams produce significantly larger families surviving to maturity. Secondly, in most families the more heterozygous individuals survive in excess of the expected mendelian proportions.

THE HISTORY OF AN INBRED LINE

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The progeny of a single female *Drosophila subobscura*, caught and fertilised in the wild, have been brother-sister mated for ten generations. In each generation fast and slow developing flies have been selected and mated together.

There has been no advance under selection, the progeny of slow pairs developing as fast or faster than the progeny of fast pairs. However, sterility has appeared first in the slow selected lines, and five such lines have died out.

From the F_5—F_8 less than half the pairs set up in all lines left progeny. In these generations only 25 per cent. of the eggs laid by fertilised females hatched, as compared with 100 per cent. of eggs laid by wild-caught females or their F_1 progeny. Mating such F_1 males to F_8 females gave eggs all of which hatched, as compared with a 56 per cent. hatch of eggs from F_1 females fertilised by F_8 males. The sterility of the inbred line is therefore mainly due to the inadequacy of the sperm produced by inbred males.

No satisfactory explanation of these facts can yet be put forward, and the investigation is continuing.

THE TRUNCATED NEGATIVE BINOMIAL DISTRIBUTION

M. R. SAMPFORD
Design and Analysis of Scientific Experiment, Oxford

The negative binomial distribution, for which the probability of observing r counts is the coefficient of t in the expansion of

$$\left\{(1+p)-pt\right\}^{-k} \qquad (1)$$

(p and k positive), is extensively used in biology for the description of data too variable to be fitted by a Poisson distribution. In particular it has been found to fit, in certain cases, the proportions of cells in irradiated tissue with O, 1, 2 . . . chromosome breaks. A difficulty arises in fitting the distribution when only some cells, or only cells in certain phases of the mitotic cycle, are susceptible to breakage. In this case, only susceptible cells should be included in the negative binomial sample, but there is no way of determining whether a cell with no breaks was or was not susceptible at the time of irradiation. The sample is therefore limited to those cells showing 1, 2 . . . breaks, and these should conform to a truncated negative binomial distribution, in which the probabilities are those given by (1) for $r = 1, 2$ . . divided by $1-(1+p)^{-k}$.

Two trial and error methods for estimating k and p will be described, one depending on the mean and variance of the truncated sample, the other leading to maximum likelihood estimates.

EVIDENCE FOR A LINEAR ORDER OF CERTAIN TRANSDUCIBLE GENES IN *SALMONELLA*

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Hereditary properties, and by inference genes, may be *transduced* by phage from one *Salmonella* strain to another. A *Salmonella* strain produces flagella only if it carries the wild-type form of each of a series of *Fla* genes. In general a cell does not simultaneously acquire by transduction more than one new character. However certain non-flagellated strains when they acquire flagellation by transduction of

an *Fla*+ gene sometimes also acquire a flagellar antigen from the *gene-donor* strain; this is attributed to the occasional simultaneous transfer of an antigen-determining gene (H_1) linked to the *Fla*+ gene being transduced.

Three different H_1- linked *Fla*− genes respectively cause absence of flagella in three strains which have different (latent) flagellar antigens. If the transducible genes of *Salmonella* are linearly arranged and if in transduction only one linear fragment replaces its homologue, then the linear order of the four loci concerned can be inferred from the observed interactions of the three strains. The argument is, *e.g.*, that phage grown on an Fla_1^+ ———Fla_2^- ———H_1^a strain cannot evoke flagellated clones with antigen *a* from an Fla_1^- ———Fla_2^+———H_1^b strain, since transduction of the terminal genes would bring in the central *Fla*− gene, which would suppress flagellation. Certain predictions from the inferred gene order have been verified by experiment.

GENETIC CONTROL OF PRODUCTION OF AN ENZYME IN *NEUROSPORA*

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It has previously been reported that a mutant of *Neurospora* with a growth requirement for α-amino nitrogen lacks the enzyme glutamic acid dehydrogenase. This observation has been confirmed and extended. The enzyme is produced by the wild-type under a variety of cultural conditions, although its level depends to some extent on the nitrogen source. The enzyme has never been detected in the mutant under any conditions of growth even though less than 1 per cent. of normal activity should have been detectable. Experiments on enzyme production by heterokaryons show that mutant nuclei have no depressing effect on the production of enzyme under the influence of the wild-type allele. The mutation seems to have no effect on the level of other enzymes related to glutamic acid dehydrogenase in their substrate specificities. The data are consistent with the view that the gene, at least in this case, is concerned rather directly with the synthesis of a single enzyme.

IRRADIATION OF *SPIROGYRA* CHROMOSOMES

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It was suggested in a paper read to this Society in 1950 that the long chromosomes of this organism were compound and polycentric, rather than possessed of the so-called "diffuse centromere," said to be found in other organisms whose chromosomes behave similarly. These long chromosomes have now been irradiated by Mr Flanders of the Medical Research Unit, Hammersmith Hospital. After receiving 3000 r each nucleus showed fragments, the mean number per nucleus being 6. The material given this dose on 15th December 1953, is still surviving and carrying out mitosis; the fragments are still there. After receiving 15,000 r approximately 90 fragments were found. They passed to the mitotic poles; the material survived nine days. At this degree of fragmentation an occasional chromosome fragment was left on the equator of the mitotic spindle. This suggests the presence of non centromeric material in the chromosomes, which the hypothesis of their compound nature requires. The species was *S. crassa*, $n = 12$.

CHANGES IN MUTATION RESPONSE TO X-RAYS

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When irradiated ♂♂ are mated to a succession of 3 virgin ♀♀ every 3 days, mutation rate rises to a peak in the second brood and subsequently falls off again. In order to correlate this brood pattern of mutation rates with the underlying sensitivity pattern of the testis, certain effects which are known to be produced in definite developmental stages were used as landmarks. These were : (1) excessive sterility, due to lack of sperm from irradiated early spermatocytes ; (2) the first cross-overs, produced by meiotic crossing-over ; (3) bunches of identical or complementary cross-overs, produced by spermatogonial crossing-over ; (4) germinal selection, limited to early, not yet cyst-forming spermatogonia. The following sensitivity pattern was derived : sensitivity is low in spermatogonia, even when germinal selection has ceased to operate ; it rises steeply during meiosis and reaches a peak at some time during spermiogenesis ; in mature sperm it has again dropped to about the level held in early meiosis. The sensitivity patterns to mustard gas and X-rays are superficially very similar ; whether they are identical can only be determined by a more thorough analysis of the mustard gas pattern. In regard to other mutagens other sensitivity patterns have been found. Knowledge of sensitivity patterns is indispensable for quantitative work with mutagens ; it may also throw light on their mode of action.

CORRELATION BETWEEN BIVALENTS IN CHIASMA FREQUENCY IN *ENDYMION NON-SCRIPTUS*

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Correlation between bivalents in chiasma frequency has been investigated in plants of diverse mean total chiasmata per cell in *Endymion non-scriptus* (*Scilla non-scripta*) (Liliaceæ). Positive and negative correlation, and absence of correlation, have been found. This type of correlation observed is not related to the mean chiasma frequency per cell, but to the amount of internuclear variation.

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Sánchez-Monge, E.

SWEDEN
Bonnier, G.
Hansson, A.
Müntzing, A.

TASMANIA
Barber, H. N.
Dobie, D. L.
Ford, J. M.
McAulay, A. L.
Sharman, G. B.

UGANDA
Hutchinson, J. B.

U.S.A.
Dempster, E. R.
Dobzhansky, Th.
Epling, C.
Haskins, C. P.
Haskins, E. F.
Lederberg, J.
Lerner, I. M.
Mainland, G. B.
Oster, I. I.
Shultz, F. T.
Snell, G. D.

UNITED KINGDOM
Anderson, D.
Bailey, N. J. J.
Bateman, A. J.
Behr, I.
Bennett, J. H.
Biggar, W. S.

UNITED KINGDOM—contd.
Billingham, R.
Blyth, J. S. S.
Cain, A. J.
Cavalli, L. L.
Cocks, B.
Crane, M. B.
Crowe, L. K.
Daday, H.
Dawson, G. W. P.
Donald, H. P.
Darlington, C. D.
Dowdeswell, W. H.
Dowrick, G. J.
Falconer, D. S.
Finney, D. J.
Fisher, R. A.
Ford, E. B.
Fyfe, D. S.
Greenwood, A. W.
Hair, J. B.
Harrison, B. J.
Haskell, G.
Hayman, B. I.
Holt, H. A.
Hurcombe, R.
Ikin, E. W.
Jinks, J. L.
Koller, P. C.
La Cour, L. F.
Lampkin, G. H.
Lane, G. R.
Lewis, D.
Logan, D. M.
Maccacaro, G. A.
Mather, K.
Medawar, P. B.
Morrison, J. W.
Mourant, A. E.
Muldal, S.
Nelder, J. A.
Nutman, P. S.
Owen, A. R. G.
Race, R. R.
Rees, H.
Sanger, R.
Searle, A. G.
Sheppard, P. M.
Skellam, J. G.
Slope, D.
Snoad, B.
Stanton, W. R.
Thompson, J. S.
Vines, A.
Wallace, M. E.
Watkin, I. M.
Williams, W.
Wylie, A. P.
Yates, F.

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CONTENTS

PAGE

MULLER, H. J., ALTENBURG, LUOLIN S., MEYER, HELEN UNGER, EDMONDSON, MARGARET, and ALTENBURG, EDGAR (Bloomington). The lack of proportionality between mutation rate and ultraviolet dose in *Drosophila* 153

FISHER, SIR RONALD (Cambridge). A fuller theory of "junctions" in inbreeding 187

BONNIER, G. (Stockholm). Secondary influences from X-ray irradiation on mutational processes in *Drosophila melanogaster* 199

LÜNING, K. G. (Stockholm). Variations in the breakability of chromosomes in mature spermatozoa of *Drosophila melanogaster* at different modes of irradiation 211

HUTCHINSON, J. B. (Uganda). New evidence on the origin of the old world cottons 225

LEUPOLD, U., and HOTTINGUER, H. (Paris). Some data on segregation in *Saccharomyces* 243

CLIFFORD, H. T. (Melbourne). Analysis of suspected hybrid swarms in the genus *Eucalyptus* 259

SNELL, G. D., DICKIE, M. M., SMITH, P., and KELTON, D. E. (Maine). Linkage of loop-tail, leaden, splotch and fuzzy in the mouse 271

BUTLER, L. (Toronto). The effect of the coat colour dilution gene on body size in the mouse . . . 275

REVIEWS :

DARLINGTON, C. D. The Facts of Life . . 279

WIENER, A. S. Rh-Hr blood types 282

WIENER, A. S. An Rh-Hr syllabus 285

CAIN, A. J. Animal species and their evolution . 286

KALLMANN, F. J. Heredity in health and mental disorder 288

GLASS, D. V. (ed.). Introduction to Malthus . 288

BUCHHOLZ, A. Ideologie und Forschung in der Sowjetischen Naturwissenschaft 289

HERSKOWITZ, I. H. Bibliography on the genetics of *Drosophila*, Part 2 289

DARLINGTON, C. D. The place of botany in the life of a university 290

GENETICAL SOCIETY OF GREAT BRITAIN : Abstracts of Papers 291